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<b>(21) International Application Number:</b> PCT/US99/16424 <b>(22) International Filing Date:</b> 21 July 1999 (21.07.99) <b>(30) Priority Data:</b> 60/093,643 21 July 1998 (21.07.98) US <b>(71) Applicant (for all designated States except US):</b> HUMAN GENOME SCIENCES, INC. [US/US]; 9410 Key West Avenue, Rockville, MD 20850 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> RUBEN, Steven, M. [US/US]; 18528 Heritage Hills Drive, Olney, MD 20832 (US). MOORE, Paul, A. [US/US]; 19005 Leatherbark Drive, Germantown, MD 20874 (US). LAFLEUR, David, W. [US/US]; 3142 Quesada Street, N.W., Washington, DC 20015 (US). <b>(74) Agents:</b> BROOKES, A., Anders et al.; Human Genome Sciences, Inc., 9410 Key West Avenue, Rockville, MD 20850 (US).	<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>	
<b>(54) Title:</b> KERATINOCYTE DERIVED INTERFERON  <b>(57) Abstract</b>  The present invention relates to a novel KDI protein which is a member of the interferon family. In particular, isolated nucleic acid molecules are provided encoding a human interferon polypeptide, called "KDI". KDI polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of KDI activity. Also provided are therapeutic methods for treating immune system-related disorders.		

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## Keratinocyte Derived Interferon

### *Field of the Invention*

5 The present invention relates to a novel human gene encoding a polypeptide which is a member of the interferon family. More specifically, isolated nucleic acid molecules are provided encoding a human polypeptide named "Keratinocyte Derived Interferon" or "KDI". KDI polypeptides are also provided, as are vectors, host cells and recombinant methods for  
10 producing the same. Also provided are diagnostic methods for detecting disorders related to the immune system, and therapeutic methods for treating disorders of the immune system. The invention further relates to screening methods for identifying agonists and antagonists of KDI.

### *Background of the Invention*

15 Interferons (IFNs) are a well known family of cytokines secreted by a large variety of eukaryotic cells upon exposure to various stimuli. The interferons have been classified by their chemical and biological characteristics into four groups: IFN-alpha (leukocytes), IFN-beta (fibroblasts), IFN-gamma (lymphocytes), and IFN-omega (leukocytes). IFN-alpha and beta are known as Type I interferons; IFN-gamma is known as a Type-II or immune interferon. A single functional gene in the human genome codes for interferon omega (IFN-omega), a  
20 monomeric glycoprotein distantly related in structure to IFN-alpha and IFN-beta, but unrelated to IFN-gamma. IFN-omega is secreted by virus-infected leukocytes as a major component of human leukocyte interferon. The IFNs exhibit anti-viral, immunoregulatory, and antiproliferative activity. The clinical potential of interferons has been recognized, and will be summarized below.

25 *Anti-viral:* IFNs have been used clinically for anti-viral therapy, for example, in the treatment of AIDS (Lane, *Semin. Oncol.* 18:46-52 (Oct. 1991)), viral hepatitis including chronic hepatitis B, hepatitis C (Woo, M.H. and Brunakis, T.G., *Ann. Pharmacother.* 31:330-337 (March 1997); Gibas, A.L., *Gastroenterologist*, 1:129-142 (June 1993)), hepatitis D, papilloma viruses (Levine, L.A. et al., *Urology* 47:553-557 (April 1996)), herpes (Ho, M., *Annu. Rev. Med.* 38:51-59 (1987)), viral encephalitis (Wintergerst et al., *Infection*, 20:207-212 (July 1992)), and in the prophylaxis of rhinitis and respiratory infections (Ho, M., *Annu. Rev. Med.* 38:51-59 (1987)).

*Anti-parasitic:* IFNs have been suggested for anti-parasite therapy, for example, IFN-gamma for treating *Cryptosporidium parvum* infection (Rehg, J.E., *J. Infect. Dis.* 174:229-232 (July 1996)).

5       *Anti-bacterial:* IFNs have been used clinically for anti-bacterial therapy. For example, IFN-gamma has been used in the treatment of multidrug-resistant pulmonary tuberculosis (Condos, R. *et al.*, *Lancet* 349:1513-1515 (1997)).

10       *Anti-cancer:* Interferon therapy has been used in the treatment of numerous cancers (e.g., hairy cell leukemia (Hofmann *et al.*, *Cancer Treat. Rev.* 12 (Suppl. B):33-37 (Dec. 1985)), acute myeloid leukemia (Stone, R.M. *et al.* *Am. J. Clin. Oncol.* 16:159-163 (April 1993)), osteosarcoma (Strander, H. *et al.*, *Acta Oncol.* 34:877-880 (1995)), basal cell carcinoma (Dogan, B. *et al.*, *Cancer Lett.* 91:215-219 (May 1995)), glioma (Fetell, M.R. *et al.*, *Cancer* 65: 78-83 (Jan. 1990)), renal cell carcinoma (Aso, Y. *et al.* *Prog. Clin. Biol. Res.* 303:653-659 (1989)), multiple myeloma (Peest, D. *et al.*, *Br. J. Haematol.* 94:425-432 (Sept. 1996)), melanoma (Ikic, D. *et al.*, *Int. J. Dermatol.* 34:872-874 (Dec. 1995)), and Hodgkin's disease (Rybak, M.E. *et al.*, *J. Biol. Response Mod.* 9:1-4 (Feb. 1990)). Synergistic treatment of advanced cancer with a combination of alpha interferon and temozolomide has also been reported (Patent publication WO 9712630 to Dugan, M.H.).

20       *Immunotherapy:* IFNs have been used clinically for immunotherapy or more particularly, (1) for example, to prevent graft vs. host rejection, or to curtail the progression of autoimmune diseases, such as arthritis, multiple sclerosis, (2) or diabetes (3). IFN-beta is approved of sale in the United States for the treatment (i.e., as an immunosuppressant) of multiple sclerosis. Recently it has been reported that patients with multiple sclerosis have diminished production of type I interferons and interleukin-2 (Wandinger, K.P. *et al.*, *J. Neurol. Sci.* 149: 87-93 (1997)). In addition, immunotherapy with recombinant IFN-alpha (in combination with recombinant human IL-2) has been used successfully in lymphoma patients following autologous bone marrow or blood stem cell transplantation, that may intensify remission following translation (Nagler, A. *et al.*, *Blood* 89: 3951-3959 (June 1997)).

35       *Anti-allergy:* The administration of IFN-gamma has been used in the treatment of allergies in mammals (See, Patent Publication WO 8701288 to Parkin, J.M. and Pinching, A.J.). It has also recently been demonstrated that there is a reduced production of IL-12 and IL-12-dependent IFN-gamma release in patients with allergic asthma (van der Pouw Kraan, T.C. *et al.*, *J. Immunol.* 158:5560-5565 (1997)). Thus, IFN may be useful in the treatment of allergy by inhibiting the humoral response.

*Vaccine adjuvantation:* Interferons may be used as an adjuvant or coadjuvant to enhance or simulate the immune response in cases of prophylactic or therapeutic vaccination (Heath, A.W. and Playfair, J.H.L., *Vaccine* 10:427-434 (1992)).

Clearly, there exists a need in the art for the discovery of novel interferon proteins for numerous applications, in *e.g.*, immunotherapy, as well as anti-viral, anti-parasitic, anti-bacterial, or anti-cancer therapies, or any medical condition or situation where increased interferon activity is desired.

### *Summary of the Invention*

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding at least a portion of the KDI polypeptide having the complete amino acid sequence shown in SEQ ID NO:2 or the complete amino acid sequence encoded by the cDNA clone deposited as plasmid DNA as ATCC Deposit Number 203500 on December 1, 1998. The nucleotide sequence determined by sequencing the deposited KDI clone (HKAPI15) which is shown in Figure 1 (SEQ ID NO:1), contains an open reading frame encoding a complete polypeptide of 207 amino acid residues, including an initiation codon encoding an N-terminal methionine at nucleotide positions 35-37. Nucleic acid molecules of the invention include those encoding the complete amino acid sequence excepting the N-terminal methionine shown in SEQ ID NO:2, which molecules also can encode additional amino acids fused to the N-terminus of the KDI amino acid sequence.

The encoded polypeptide has a predicted leader sequence of 27 amino acids underlined in Figure 1; and the amino acid sequence of the predicted mature KDI protein is also shown in Figure 1 as amino acid residues 28-207 and as residues 1-207 in SEQ ID NO:2.

Residues 165 to 183 of Figure 1 (SEQ ID NO:2) comprise the signature sequence for interferon polypeptides, and the KDI polypeptide in particular. Thus, preferred are polypeptides comprising residues 165 to 183 of Figure 1 as are polynucleotides encoding such polypeptides.

Thus, one aspect of the invention provides an isolated nucleic acid molecule comprising a polynucleotide comprising a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding the KDI polypeptide having the complete amino acid sequence in SEQ ID NO:2; (b) a nucleotide sequence encoding the KDI polypeptide having the complete amino acid sequence in SEQ ID NO:2 excepting the N-terminal methionine (*i.e.*, residues 2-207 of SEQ ID NO:2); (c) a nucleotide sequence encoding the mature KDI polypeptide shown as residues 28-207 in SEQ ID NO:2; (d) a nucleotide sequence encoding residues 165-183 of SEQ ID NO:2; (e) a nucleotide sequence encoding the complete polypeptide encoded by the human cDNA contained in clone HKAPI15; (f) a nucleotide sequence encoding the complete polypeptide encoded by the human cDNA contained in clone HKAPI15 excepting the N-

terminal methionine; (g) a nucleotide sequence encoding the mature polypeptide encoded by the human cDNA contained in clone HKAPI15; and (h) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f) or (g) above.

Further embodiments of the invention include isolated nucleic acid molecules that  
5 comprise a polynucleotide having a nucleotide sequence at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical, to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g) or (h), above, or a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide in (a), (b), (c), (d), (e), (f), (g) or (h),  
10 above. This polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues. An additional nucleic acid embodiment of the invention relates to an isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a KDI polypeptide having an amino acid sequence in (a), (b), (c),  
15 (d), (e), (f) or (g), above.

The present invention also relates to recombinant vectors, which include the isolated  
nucleic acid molecules of the present invention, and to host cells containing the recombinant  
vectors, as well as to methods of making such vectors and host cells and for using them for  
production of KDI polypeptides or peptides by recombinant techniques.

The invention further provides an isolated KDI polypeptide comprising an amino acid  
20 sequence selected from the group consisting of: (a) the amino acid sequence of the full-length KDI polypeptide having the complete amino acid sequence shown in SEQ ID NO:2; (b) the amino acid sequence of the full-length KDI polypeptide having the complete amino acid sequence shown in SEQ ID NO:2 excepting the N-terminal methionine (i.e., residues 2 to 207 of SEQ ID NO:2); the amino acid sequence of the mature KDI polypeptide shown as residues  
25 28-207 in SEQ ID NO:2; (d) the amino acid sequence shown as residues 165 to 183 of SEQ ID NO:2; (e) the full length KDI polypeptide encoded by the human cDNA contained in clone HKAPI15; (f) the full-length KDI polypeptide encoded by the human cDNA contained in clone HKAPI15 excepting the N-terminal methionine; and (g) the mature KDI polypeptide encoded by the human cDNA contained in clone HKAPI15. The polypeptides of the present invention  
30 also include polypeptides having an amino acid sequence at least 80% identical, more preferably at least 90% identical, and still more preferably 95%, 96%, 97%, 98% or 99% identical to those described in (a), (b), (c), (d), (e), (f) or (g) above, as well as polypeptides having an amino acid sequence with at least 90% similarity, and more preferably at least 95% similarity, to those  
above.

35 An additional embodiment of this aspect of the invention relates to a peptide or polypeptide which comprises the amino acid sequence of an epitope-bearing portion of a KDI polypeptide having an amino acid sequence described in (a), (b), (c), (d), (e), (f), or (g), above. Peptides or polypeptides having the amino acid sequence of an epitope-bearing portion

of a KDI polypeptide of the invention include portions of such polypeptides with at least six or seven, preferably at least nine, and more preferably at least about 30 amino acids to about 50 amino acids, although epitope-bearing polypeptides of any length up to and including the entire amino acid sequence of a polypeptide of the invention described above also are included in the invention.

In another embodiment, the invention provides an isolated antibody that binds specifically to a KDI polypeptide having an amino acid sequence described in (a), (b), (c), (d), (e), (f) or (g) above. The invention further provides methods for isolating antibodies that bind specifically to a KDI polypeptide having an amino acid sequence as described herein. Such antibodies are useful therapeutically as described below.

The invention also provides for pharmaceutical compositions comprising KDI polypeptides which may be employed, for instance, to treat immune system-related disorders such as viral infection, parasitic infection, bacterial infection, cancer, autoimmune disease, multiple sclerosis, lymphoma and allergy. Methods of treating individuals in need of interferon polypeptides are also provided.

The invention further provides compositions comprising a KDI polynucleotide or a KDI polypeptide for administration to cells *in vitro*, to cells *ex vivo* and to cells *in vivo*, or to a multicellular organism. In certain particularly preferred embodiments of this aspect of the invention, the compositions comprise a KDI polynucleotide for expression of a KDI polypeptide in a host organism for treatment of disease. Particularly preferred in this regard is expression in a human patient for treatment of a dysfunction associated with aberrant endogenous activity of an interferon.

The present invention also provides a screening method for identifying compounds capable of enhancing or inhibiting a biological activity of the KDI polypeptide, which involves contacting a receptor which is enhanced by the KDI polypeptide with the candidate compound in the presence of a KDI polypeptide, assaying, for example, anti-viral activity in the presence of the candidate compound and the KDI polypeptide, and comparing the activity to a standard level of activity, the standard being assayed when contact is made between the receptor and KDI in the absence of the candidate compound. In this assay, an increase in activity over the standard indicates that the candidate compound is an agonist of KDI activity and a decrease in activity compared to the standard indicates that the compound is an antagonist of KDI activity.

It has been discovered that KDI is expressed in keratinocytes. Therefore, nucleic acids of the invention are useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). In addition, for a number of disorders of the above tissues or cells, particularly of the immune system, significantly higher or lower levels of KDI gene expression may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g.,

serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" KDI gene expression level, i.e., the KDI expression level in healthy tissue from an individual not having the immune system disorder. Thus, the invention provides a diagnostic method useful during diagnosis of such a disorder, which involves: (a) assaying KDI gene expression level in cells or body fluid of an individual; (b) comparing the KDI gene expression level with a standard KDI gene expression level, whereby an increase or decrease in the assayed KDI gene expression level compared to the standard expression level is indicative of disorder in the immune system.

An additional aspect of the invention is related to a method for treating an individual in need of an increased level of interferon activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an isolated KDI polypeptide of the invention or an agonist thereof.

A still further aspect of the invention is related to a method for treating an individual in need of a decreased level of interferon activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of a KDI antagonist. Preferred antagonists for use in the present invention are KDI-specific antibodies.

### ***Brief Description of the Figures***

Figure 1 shows the nucleotide sequence (SEQ ID NO:1) and amino acid sequence (SEQ ID NO:2) of KDI.

Figure 2 shows the regions of identity between the amino acid sequences of the KDI protein and translation product of the human mRNA for Interferon Omega (SEQ ID NO:3), determined by the computer program Bestfit (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711).

Figure 3 shows an analysis of the KDI amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index - Jameson-Wolf" graph, the positive peaks indicate locations of the highly antigenic regions of the KDI protein, i.e., regions from which epitope-bearing peptides of the invention can be obtained.

Figure 4 shows an alignment of the KDI polypeptide of the present invention with several other members of the interferon polypeptide family. Shown is human interferon beta-1 (SEQ ID NO:4), human placental interferon (SEQ ID NO:5), human interferon omega (SEQ ID NO:6), human interferon alpha-c (SEQ ID NO:7), human interferon alpha-F (SEQ ID NO:8), human interferon II-1 (SEQ ID NO:9), human alpha interferon-N (SEQ ID NO:10), bovine TP-1 (SEQ ID NO:11), ovi TP-1 (SEQ ID NO:12), pig TP (SEQ ID NO:13), human interferon beta 2a (IL-6) (SEQ ID NO:14), bovine interferon beta-2 (SEQ ID NO:15), bovine interferon beta-1 (SEQ ID NO:20), synthetic interferon beta-1 (SEQ ID NO:21). The alignment was produced



by the Megalign routine using the Clustal method with PAM250 residue weight table. Megalign is contained within the DNASTar suite of programs.

### ***Detailed Description***

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a Keratinocyte-Derived Interferon polypeptide (hereinafter "KDI") having the amino acid sequence shown in SEQ ID NO:2. The nucleotide sequence shown in Figure 1 (SEQ ID NO:1) was obtained by sequencing the human HKAPI15 cDNA clone which was deposited on December 1, 1998 at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, USA, and given accession number ATCC 203500. The deposited cDNA is contained in the plasmid pCMVSPORT 2.0 (Life Technologies, Gaithersburg MD) and can be excised by the SalI/NotI restriction enzyme sites flanking the human cDNA.

The KDI protein of the present invention shares sequence homology with many members of the interferon family, notably the translation product of the human mRNA for IFN-omega (Figure 2) (SEQ ID NO:3). IFN-omega has been shown to inhibit the proliferation of a variety of tumor cell lines in vitro, stimulate natural killer cell activity, enhance expression of major histocompatibility complex class I (but not class II) antigens and inhibit proliferation of lymphocytes stimulated with mitogens or allogeneic cells. Adolf, G.R., Human interferon omega--a review, *Mult Scler* 1995;1 Suppl 1:S44-S47. The expression of KDI has been determined to be primarily found in keratinocytes, dendritic cells and monocytes, but is particularly strong in keratinocytes. Stimulation of keratinocytes with TNF- $\alpha$  or PolyIC (simulating viral infection) specifically and rapidly stimulates overexpression of the KDI transcript. Based on its structural similarity to IFN-omega and its increased expression in response to simulated viral infection, KDI is believed to share many of its biological activities of INF-Omega and other interferon proteins, including, inhibition of tumor proliferation, antiviral activities, NK cell activation, and immune system enhancement.

### ***Nucleic Acid Molecules***

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer (such as the Model 373 from Applied Biosystems, Inc., Foster City, CA), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of

the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

By "nucleotide sequence" of a nucleic acid molecule or polynucleotide is intended, for a DNA molecule or polynucleotide, a sequence of deoxyribonucleotides, and for an RNA molecule or polynucleotide, the corresponding sequence of ribonucleotides (A, G, C and U), where each thymidine deoxyribonucleotide (T) in the specified deoxyribonucleotide sequence is replaced by the ribonucleotide uridine (U).

Using the information provided herein, such as the nucleotide sequence in Figure 1 (SEQ ID NO:1), a nucleic acid molecule of the present invention encoding a KDI polypeptide may be obtained using standard molecular biology procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in Figure 1 (SEQ ID NO:1) was discovered in a cDNA library derived from isolated keratinocytes.

The nucleotide sequence of the KDI DNA of Figure 1 (SEQ ID NO:1) contains an open reading frame encoding a protein of 207 amino acid residues, with an initiation codon at nucleotide positions 35-37 of the nucleotide sequence in Figure 1 (SEQ ID NO:1). The amino acid sequence of the KDI protein shown in SEQ ID NO:2 is about 35% identical to IFN-omega, (Figure 2). The sequences of INF-Omega can be accessed through GenBank with Accession No. gb|A12140.

As one of ordinary skill would appreciate, due to the possibilities of sequencing errors discussed above, the actual complete KDI polypeptide encoded by the deposited cDNA, which comprises about 207 amino acids, may be somewhat longer or shorter. More generally, the actual open reading frame may be anywhere in the range of  $\pm 20$  amino acids, more likely in the range of  $\pm 10$  amino acids, of that predicted from the methionine codon at the N-terminus shown in Figure 1 (SEQ ID NO:1).

### ***Leader and Mature Sequences***

The amino acid sequence of the complete KDI protein includes a leader sequence and a mature protein, as shown in SEQ ID NO:2. More in particular, the present invention provides nucleic acid molecules encoding a mature form of the KDI protein. Thus, according to the signal hypothesis, once export of the growing protein chain across the rough endoplasmic reticulum has been initiated, proteins secreted by mammalian cells have a signal or secretory

leader sequence which is cleaved from the complete polypeptide to produce a secreted "mature" form of the protein. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide. Therefore, the present invention provides a nucleotide sequence encoding the mature KDI polypeptide having the amino acid sequence encoded by the human cDNA in clone HKAPI15 (ATCC Deposit No. 203500). By the "mature KDI polypeptide having the amino acid sequence encoded by the human cDNA in clone HKAPI15" is meant the mature form(s) of the KDI protein produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the human DNA sequence of the clone contained in the deposited vector.

In addition, methods for predicting whether a protein has a secretory leader as well as the cleavage point for that leader sequence are available. For instance, the method of McGeoch (*Virus Res.* 3:271-286 (1985)) uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje (*Nucleic Acids Res.* 14:4683-4690 (1986)) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2 where +1 indicates the amino terminus of the mature protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80% (von Heinje, *supra*). However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the deduced amino acid sequence of the complete KDI polypeptide was analyzed by a computer program "PSORT", available from Dr. Kenta Nakai of the Institute for Chemical Research, Kyoto University (see K. Nakai and M. Kanehisa, *Genomics* 14:897-911 (1992)), which is an expert system for predicting the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated." The computation analysis above predicted one potential cleavage site within the complete amino acid sequence shown in SEQ ID NO:2; that is, between residues 27 and 28 in Figure 1 (SEQ ID NO:2). Of course the exact address of the cleavage site used by naturally occurring enzymes may vary slightly from the predicted cleavage site and may vary between species. Accordingly, mature polypeptides beginning from about residue 20 to about residue 34 are provided. More in particular, the invention provides a polypeptide having a portion of SEQ ID NO:2 as follows: residues 20-207 in SEQ ID NO:2, residues 21-207 in SEQ ID NO:2, residues 22-207 in SEQ ID NO:2, residues 23-207 in SEQ ID NO:2, residues 24-207 in SEQ ID NO:2, residues 25-207 in SEQ ID NO:2, residues 26-207 in SEQ ID NO:2, residues 27-207 in SEQ ID NO:2, residues 28-207 in SEQ ID NO:2, residues

29-207 in SEQ ID NO:2, residues 30-207 in SEQ ID NO:2, residues 31-207 in SEQ ID NO:2, residues 32-207 in SEQ ID NO:2, residues 33-207 in SEQ ID NO:2, and residues 34-127 in SEQ ID NO:2. The invention also provides polynucleotides encoding such polypeptides.

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, or in the form of DNA. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

In specific embodiments, the polynucleotides of this invention are less than 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb or 7.5 kb in length. In a further embodiment, polynucleotides of the invention comprise at least 15 contiguous nucleotides of KDI coding sequence, but do not comprise all or a portion of any KDI intron. In another embodiment, the nucleic acid comprising KDI coding sequence does not contain coding sequences of a genomic flanking gene (i.e. 5' or 3' to the KDI coding sequence in the genome).

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. However, a nucleic acid contained in a clone that is a member of a library (e.g., a genomic or cDNA library) that has not been isolated from other members of the library (e.g., in the form of a homogenous solution containing the clone and other members of the library) or a chromosome isolated or removed from a cell or cell lysate (e.g., a "chromosome spread", as in a karyotype), is not "isolated" for the purposes of this invention. As discussed further herein, isolated nucleic acid molecules according to the present invention may be produced naturally, recombinantly or synthetically.

Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) with an initiation codon at positions 35-37 of the nucleotide sequence shown in Figure 1 (SEQ ID NO:1).

Also included are DNA molecules comprising the coding sequence for the KDI protein lacking an N-terminal methionine shown at positions 2-207 of SEQ ID NO:2.

In addition, isolated nucleic acid molecules of the invention include DNA molecules which comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode the KDI protein. Of course, the genetic code and species-specific codon preferences are well known in the art. Thus, it would be routine for one skilled in the art to generate the degenerate variants described above, for instance, to optimize codon expression for a particular host (e.g., change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

In another aspect, the invention provides isolated nucleic acid molecules encoding the KDI polypeptide having an amino acid sequence encoded by the human cDNA in clone HKAPI15 (ATCC Deposit No. 203500). Preferably, this nucleic acid molecule will encode the mature polypeptide encoded by the above-described deposited human cDNA.

5 The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in Figure 1 (SEQ ID NO:1) or the nucleotide sequence of the KDI cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful for production of the KDI polypeptide of the invention and as a probe for  
10 detection of mRNA in cells transfected with a vector for the purpose of producing KDI; i.e., as a marker for determining expression of the heterologous gene in a host cell.

The present invention is further directed to nucleic acid molecules encoding portions of the nucleotide sequences described herein as well as to fragments of the isolated nucleic acid molecules described herein. In particular, the invention provides a polynucleotide having a  
15 nucleotide sequence representing the portion of SEQ ID NO:1 which consists of positions 35-655 of SEQ ID NO:1. Other particularly preferred polynucleotide fragments of the invention comprise, or alternatively, consist of nucleotide residues 38-655, 41-655, 44-655, 47-655, 50-655, 53-655, 56-655, 59-655, 62-655, 65-655, 68-655, 71-655, 74-655, 77-655, 80-655, 83-655, 86-655, 89-655, 92-655, 95-655, 98-655, 101-655, 104-655, 107-655, 110-655, 113-655, 116-655, 119-655, 122-655, 125-655, 128-655, 131-655, 134-655, 137-655, 140-655,  
20 143-655, 146-655, 149-655, 152-655, 155-655, 158-655, 161-655, 164-655, 167-655, 170-655, 173-655, 176-655, 179-655, 182-655, 185-655, 188-655, 191-655, 194-655, 197-655, 200-655, 203-655, 206-655, 209-655, 212-655, 215-655, 218-655, 221-655, 224-655, 227-655, 230-655, 233-655, 236-655, 239-655, 242-655, 245-655, 248-655, 251-655, 254-655, 257-655, 260-655, 263-655, 266-655, 269-655, 272-655, 275-655, 278-655, 281-655, 284-655, 287-655, 290-655, 293-655, 296-655, 299-655, 302-655, 305-655, 308-655, 311-655, 314-655, 317-655, 320-655, 323-655, 326-655, 329-655, 332-655, 335-655, 338-655, 341-655, 344-655, 347-655, 350-655, 353-655, 356-655, 359-655, 362-655, 365-655, 368-655, 371-655, 374-655, 377-655, 380-655, 383-655, 386-655, 389-655, 392-655, 395-655, 398-655, 401-655, 404-655, 407-655, 410-655, 413-655, 416-655, 419-655, 422-655, 425-655, 428-655, 431-655, 434-655, 437-655, 440-655, 443-655, 446-655, 449-655, 452-655, 455-655, 458-655, 461-655, 464-655, 467-655, 470-655, 473-655, 476-655, 479-655, 482-655, 485-655, 488-655, 491-655, 494-655, 497-655, 500-655, 503-655, 506-655, 509-655, 512-655, 515-655, 518-655, 521-655, 524-655, 527-655, 530-655, 533-655, 536-655, 539-655, 542-655, 545-655, 548-655, 551-655, 554-655, 557-655, 560-655, 563-655, 566-655, 569-655, 572-655, 575-655, 578-655, 581-655, 584-655, 587-655, 590-655, 593-655, 596-655, 599-655, 602-655, 605-655, 608-655, 611-655, 614-655, 617-655, 620-655, 623-655, 626-655, 629-655, 632-655, and 635-655 of SEQ ID NO:1. Still other particularly preferred

polynucleotide fragments of the invention comprise, or alternatively, consist of nucleotide residues 38-68, 38-71, 38-74, 38-77, 38-80, 38-83, 38-86, 38-89, 38-92, 38-95, 38-98, 38-101, 38-104, 38-107, 38-110, 38-113, 38-116, 38-119, 38-122, 38-125, 38-128, 38-131, 38-134, 38-137, 38-140, 38-143, 38-146, 38-149, 38-152, 38-155, 38-158, 38-161, 38-164, 38-167, 38-170, 38-173, 38-176, 38-179, 38-182, 38-185, 38-188, 38-191, 38-194, 38-197, 38-200, 38-203, 38-206, 38-209, 38-212, 38-215, 38-218, 38-221, 38-224, 38-227, 38-230, 38-233, 38-236, 38-239, 38-242, 38-245, 38-248, 38-251, 38-254, 38-257, 38-260, 38-263, 38-266, 38-269, 38-272, 38-275, 38-278, 38-281, 38-284, 38-287, 38-290, 38-293, 38-296, 38-299, 38-302, 38-305, 38-308, 38-311, 38-314, 38-317, 38-320, 38-323, 38-326, 38-329, 38-335, 38-338, 38-341, 38-344, 38-347, 38-350, 38-353, 38-356, 38-359, 38-362, 38-365, 38-368, 38-371, 38-374, 38-377, 38-380, 38-383, 38-386, 38-389, 38-392, 38-395, 38-398, 38-401, 38-404, 38-407, 38-410, 38-413, 38-416, 38-419, 38-422, 38-425, 38-428, 38-431, 38-434, 38-437, 38-440, 38-443, 38-446, 38-449, 38-452, 38-455, 38-458, 38-461, 38-464, 38-467, 38-470, 38-473, 38-476, 38-479, 38-482, 38-485, 38-488, 38-491, 38-494, 38-497, 38-500, 38-503, 38-506, 38-509, 38-512, 38-515, 38-518, 38-521, 38-524, 38-527, 38-530, 38-533, 38-536, 38-539, 38-542, 38-545, 38-548, 38-551, 38-554, 38-557, 38-560, 38-563, 38-566, 38-569, 38-572, 38-575, 38-578, 38-581, 38-584, 38-587, 38-590, 38-593, 38-596, 38-599, 38-602, 38-605, 38-608, 38-611, 38-614, 38-617, 38-620, 38-623, 38-626, 38-629, 38-632, and 38-635 of SEQ ID NO:1.

Further, the invention includes a polynucleotide comprising any portion of at least about 30 contiguous nucleotides, preferably at least about 50 contiguous nucleotides, of SEQ ID NO:1.

More generally, by a fragment of an isolated nucleic acid molecule having the nucleotide sequence of the deposited cDNA or the nucleotide sequence shown in Figure 1 (SEQ ID NO:1) is intended fragments at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 50-600 nt in length (fragments of 400 nt, 450 nt, 500 nt, 550 nt and 600 nt in length are specifically contemplated as are fragments of all lengths between 15 and 600 but will not be specifically recited for space considerations) are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence of the deposited cDNA or as shown in Figure 1 (SEQ ID NO:1). By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the deposited cDNA or the nucleotide sequence as shown in Figure 1 (SEQ ID NO:1) and may, of course, comprise additional nucleic acid sequences not derived from SEQ ID NO:1 (or the deposited cDNA) fused to either end of the 20+ contiguous bases from SEQ ID NO:1 or the deposited cDNA. Preferred nucleic acid fragments of the present invention

include nucleic acid molecules encoding epitope-bearing portions of the KDI polypeptide as identified in Figure 3 and described in more detail below.

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, the human cDNA in clone HKAPI15 (ATCC Deposit No. 203500). By "stringent hybridization conditions" is intended overnight incubation at 42° C in a solution comprising: 50% formamide, 5x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters twice in 0.1x SSC at about 65° C.

By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 (e.g., 50) nt of the reference polynucleotide. These are useful as diagnostic probes and primers as discussed above and in more detail below.

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNA or the nucleotide sequence as shown in Figure 1 (SEQ ID NO:1)). Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3' terminal poly(A) tract of the KDI cDNA shown in Figure 1 (SEQ ID NO:1)), or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

In specific embodiments, the polynucleotides of the invention are less than 100000 kb, 50000 kb, 10000 kb, 1000 kb, 500 kb, 400 kb, 350 kb, 300 kb, 250 kb, 200 kb, 175 kb, 150 kb, 125 kb, 100 kb, 75 kb, 50 kb, 40 kb, 30 kb, 25 kb, 20 kb, 15 kb, 7.5 kb, or 5 kb in length.

As indicated, nucleic acid molecules of the present invention which encode a KDI polypeptide may include, but are not limited to those encoding the amino acid sequence of the complete polypeptide, by itself; and the coding sequence for the complete polypeptide and additional sequences, such as those encoding an added secretory leader sequence, such as a pre-, or pro- or prepro- protein sequence.

Also encoded by nucleic acids of the invention are the above protein sequences together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals, for

example - ribosome binding and stability of mRNA; an additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities.

Thus, the sequence encoding the polypeptide may be fused to a marker sequence, such as a sequence encoding a peptide which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz *et al.*, *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein, which has been described by Wilson *et al.*, *Cell* 37: 767 (1984). As discussed below, other such fusion proteins include the KDI fused to Fc at the N- or C-terminus.

### ***Variant and Mutant Polynucleotides***

The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of the KDI protein. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Such variants include those produced by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the KDI protein or portions thereof. Also especially preferred in this regard are conservative substitutions.

Further embodiments include an isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical to a polynucleotide selected from the group consisting of: (a) a nucleotide sequence encoding the KDI polypeptide having the complete amino acid sequence in SEQ ID NO:2; (b) a nucleotide sequence encoding the KDI polypeptide having the complete amino acid sequence in SEQ ID NO:2 excepting the N-terminal methionine (i.e., residues 2-161 of SEQ ID NO:2; (c) a nucleotide sequence encoding the mature KDI polypeptide having the sequence shown as residues 28-207 in SEQ ID NO:2; (d) a nucleotide sequence encoding residues 165 to 183 shown in SEQ ID NO:2; (e) a nucleotide sequence encoding the complete amino acid sequence encoded by the human cDNA contained in clone



HKAPI15; (f) a nucleotide sequence encoding the complete amino acid sequence encoded by the human cDNA contained in clone HKAPI15 excepting the N-terminal methionine; (g) a nucleotide sequence encoding the amino acid sequence of the mature polypeptide encoded by the human cDNA contained in clone HKAPI15; and (h) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g) and (h), above.

Further embodiments of the invention include isolated nucleic acid molecules that comprise a polynucleotide having a nucleotide sequence at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical, to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g) or (h), above, or a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide in (a), (b), (c), (d), (e), (f), (g) or (h) above. This polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues. An additional nucleic acid embodiment of the invention relates to an isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a KDI polypeptide having an amino acid sequence in (a), (b), (c), (d), (e), (f) or (g), above.

The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of KDI polypeptides or peptides by recombinant techniques.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a KDI polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the KDI polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in Figure 1 or to the nucleotides sequence of the deposited cDNA clone can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575

Science Drive, Madison, WI 53711). Bestfit uses the local homology algorithm of Smith and Waterman to find the best segment of homology between two sequences (*Advances in Applied Mathematics* 2:482-489 (1981)). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. (1990) 6:237-245). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base

subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not  
5 matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The present application is directed to nucleic acid molecules at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1) or to the nucleic acid sequence of the deposited DNA, irrespective of whether they encode a  
10 polypeptide having KDI activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having KDI activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having KDI activity include, inter alia, isolating allelic variants in a cDNA  
15 library.

Preferred, however, are nucleic acid molecules having sequences at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1) or to the nucleic acid sequence of the deposited DNA which do, in fact, encode a polypeptide having KDI protein activity. By "a polypeptide having KDI activity" is intended  
20 polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the KDI protein of the invention, as measured in a particular biological assay. For example, the KDI protein of the present invention inhibits bone marrow colony formation in-vitro and can be assayed according to the method of Tiefenthaler M. *et al.* (*Interferon Cytokine Res*, 1997 Jun;17(6):327-329, incorporated herein by reference in its entirety). In addition, KDI can  
25 inhibit GM-CSF induced proliferation of the erythroleukaemic cell line TF-1 according to the assays reported by Mire-Sluis A.R. *et al.* (*J. Immunol. Methods* 1996 Sep 9;195(1-2):55-61, incorporated herein by reference). Alternatively, KDI can be assayed for classical anti-viral activity by any of several assays known to those of skill in the art, for example, in the assay reported by Sugiyama, K. *et al.* (*Yakugaku Zasshi* 1995 May;115(5):390-393).

30 The KDI protein of the present invention inhibits bone marrow proliferation and shows anti-viral activity in a dose-dependent manner in the above-described assays. Thus, "a polypeptide having KDI protein activity" includes polypeptides that also exhibit any of the same activities in the above-described assays in a dose-dependent manner. Although the degree of dose-dependent activity need not be identical to that of the KDI protein, preferably, "a  
35 polypeptide having KDI protein activity" will exhibit substantially similar dose-dependence in a given activity as compared to the KDI protein (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity relative to the reference KDI protein).

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of the deposited cDNA or the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1) will encode a polypeptide "having KDI protein activity." In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having KDI protein activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

### **Vectors and Host Cells**

The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, and the production of KDI polypeptides or fragments thereof by recombinant techniques. The vector may be, for example, a phage, plasmid, viral or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli lac*, *trp*, *phoA* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells:

fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, 293 and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc., *supra*; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis *et al.*, *Basic Methods In Molecular Biology* (1986).

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to stabilize and purify proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett *et al.*, *J. Molecular Recognition* 8:52-58 (1995) and K. Johanson *et al.*, *J. Biol. Chem.* 270:9459-9471 (1995).

The KDI protein can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction,

anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Polypeptides of the present invention include: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

### ***Polypeptides and Fragments***

The invention further provides an isolated KDI polypeptide having the amino acid sequence encoded by the deposited DNA, or the amino acid sequence in SEQ ID NO:2, or a peptide or polypeptide comprising a portion of the above polypeptides.

### ***Variant and Mutant Polypeptides***

To improve or alter the characteristics of KDI polypeptides, protein engineering may be employed. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or "muteins including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions.

### ***N-Terminal and C-Terminal Deletion Mutants***

For instance, for many proteins, including the extracellular domain of a membrane associated protein or the mature form(s) of a secreted protein, it is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function. For instance, Ron et al., *J. Biol. Chem.*, 268:2984-2988 (1993) reported modified KGF proteins that had heparin binding activity even if 3, 8, or 27 amino-terminal amino acid residues were missing. In the present case, since the protein of the invention is a member of the interferon polypeptide family, deletions of N-terminal amino acids up to the

cysteine at position 59 as shown in SEQ ID NO:2 may retain some biological activity such as antiviral activity or inhibition of bone marrow proliferation. Polypeptides having further N-terminal deletions including the Cys-59 residue in SEQ ID NO:2 would not be expected to retain such biological activities because it is known that this residue in an interferon-related polypeptide is conserved among many, if not all, members of the family as is Leucine residue immediately adjacent to it (residue 60). The cysteine residue at position 59 is thought to be required for forming a disulfide bridge to provide structural stability which is needed for receptor binding and signal transduction.

However, even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete protein generally will be retained when less than the majority of the residues of the complete protein are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the KDI shown in SEQ ID NO:2, up to the Cys-59, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues n-207 of SEQ ID NO:2, where n is an integer in the range of 1-59 and where Cys-59 is the position of the first residue from the N-terminus of the complete KDI polypeptide (shown in SEQ ID NO:2) believed to be required for activity of the KDI protein.

More in particular, the invention provides polypeptides having the amino acid sequence of residues 1-207, 2-207, 3-207, 4-207, 5-207, 6-207, 7-207, 8-207, 9-207, 10-207, 11-207, 12-207, 13-207, 14-207, 15-207, 16-207, 17-207, 18-207, 19-207, 20-207, 21-207, 22-207, 23-207, 24-207, 25-207, 26-207, 27-207, 28-207, 29-207, 30-207, 31-207, 32-207, 33-207, 34-207, 35-207, 36-207, 37-207, 38-207, 39-207, 40-207, 41-207, 42-207, 43-207, 44-207, 45-207, 46-207, 47-207, 48-207, 49-207, 50-207, 51-207, 52-207, 53-207, 54-207, 55-207, 56-207, 57-207, 58-207 and 59-207, all of SEQ ID NO:2. Polynucleotides encoding these polypeptides also are provided.

Similarly, many examples of biologically functional C-terminal deletion muteins are known. For instance, Interferon gamma shows up to ten times higher activities by deleting 8-10 amino acid residues from the carboxy terminus of the protein (Döbeli et al., *J. Biotechnology* 7:199-216 (1988)). In the present case, since the protein of the invention is a member of the interferon polypeptide family, deletions of C-terminal amino acids up to the tryptophan residue at position 183 (W-183) in SEQ ID NO:2 may retain some biological activity such as antiviral activity or inhibition of bone marrow proliferation. Polypeptides having further

C-terminal deletions including Ile-183 of SEQ ID NO:2 would not be expected to retain such biological activities because it is known that this residue in an interferon-related polypeptide is conserved among many members and is thought to be important for receptor binding and signal transduction. Furthermore, the cysteine residue at position 181 is highly conserved and known to be required for antiviral activity of members of the interferon family.

However, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete protein generally will be retained when less than the majority of the residues of the complete protein are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues from the carboxy terminus of the amino acid sequence of the KDI shown in SEQ ID NO:2, up to Trp-183 of SEQ ID NO:2, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides having the amino acid sequence of residues 1-m of the amino acid sequence in SEQ ID NO:2, where m is any integer in the range of 183-207 and residue Trp-183 is the position of the first residue from the C-terminus of the complete KDI polypeptide (shown in SEQ ID NO:2) believed to be required for activity of the KDI protein.

More in particular, the invention provides polypeptides having the amino acid sequence of residues 1-183, 1-184, 1-185, 1-186, 1-187, 1-188, 1-189, 1-190, 1-191, 1-192, 1-193, 1-194, 1-195, 1-196, 1-197, 1-198, 1-199, 1-200, 1-201, 1-202, 1-203, 1-204, 1-205, 1-206 and 1-207 of SEQ ID NO:2. Polynucleotides encoding these polypeptides also are provided.

The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues n-m of SEQ ID NO:2, where n and m are integers as described above. Furthermore, the invention provides these mutant polypeptides optionally having an N-terminal methionine. The polypeptides may therefore also be described by the formula x-n-m where X is either NH<sub>2</sub> or Met and n and m are integers as described above. Polynucleotides encoding these polypeptides are, of course, also provided.

More in particular, the invention preferably provides polypeptides having the amino acid sequence of residues: 20-183, 21-183, 22-183, 23-183, 24-183, 25-183, 26-183, 27-183, 28-183, 29-183, 30-183, 31-183, 32-183, 33-183, 34-183, 35-183, 36-183, 37-183, 38-183, 39-183, 40-183, 41-183, 42-183, 43-183, 44-183, 45-183, 46-183, 47-183, 48-183, 49-183, 50-183, 51-183, 52-183, 53-183, 54-183, 55-183, 56-183, 57-183, 58-183, 59-183, 20-184, 21-184, 22-184, 23-184, 24-184, 25-184, 26-184, 27-184, 28-184, 29-184, 30-184, 31-184,



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28-205, 29-205, 30-205, 31-205, 32-205, 33-205, 34-205, 35-205, 36-205, 37-205, 38-205, 39-205, 40-205, 41-205, 42-205, 43-205, 44-205, 45-205, 46-205, 47-205, 48-205, 49-205, 50-205, 51-205, 52-205, 53-205, 54-205, 55-205, 56-205, 57-205, 58-205, 59-205, 20-206, 21-206, 22-206, 23-206, 24-206, 25-206, 26-206, 27-206, 28-206, 29-206, 30-206, 31-206, 32-206, 33-206, 34-206, 35-206, 36-206, 37-206, 38-206, 39-206, 40-206, 41-206, 42-206, 43-206, 44-206, 45-206, 46-206, 47-206, 48-206, 49-206, 50-206, 51-206, 52-206, 53-206, 54-206, 55-206, 56-206, 57-206, 58-206 and 59-206 of SEQ ID NO:2. Each of the foregoing polypeptides may additionally include an N-terminal methionine residue. Polynucleotides encoding each of these polypeptides, with or without an N-terminal methionine residues are also are provided.

Also included are polypeptides consisting of a portion of the complete KDI amino acid sequence encoded by the human cDNA in clone HKAPI15, where this portion excludes from 1 to about 58 amino acids from the amino terminus of the complete amino acid sequence encoded by the human cDNA in clone HKAPI15, or from 1 to about 23 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the human cDNA in clone HKAPI15. Polynucleotides encoding all of the above deletion mutant polypeptide forms also are provided.

### ***Other Mutants***

In addition to terminal deletion forms of the protein discussed above, it also will be recognized by one of ordinary skill in the art that some amino acid sequences of the KDI polypeptide can be varied without significant effect on the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity.

Thus, the invention further includes variations of the KDI polypeptide which show substantial KDI polypeptide activity or which include regions of KDI protein such as the protein portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions selected according to general rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science* 247:1306-1310 (1990), wherein the authors indicate that there are two main approaches for studying the tolerance of an amino acid sequence to change. The first method relies on the process of evolution, in which mutations are either accepted or rejected by natural selection. The second approach uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene and selections or screens to identify sequences that maintain functionality.

As the authors state, these studies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at a certain position of the protein. For example, most buried amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Other such phenotypically silent substitutions are described in Bowie, J. U. *et al.*,  
5 *supra*, and the references cited therein. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe, Tyr.

Thus, the fragment, derivative or analog of the polypeptide of SEQ ID NO:2, or that encoded by the deposited cDNA, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one  
15 encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the KDI polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the above form of the polypeptide, such as an IgG Fc fusion region peptide or leader or secretory  
20 sequence or a sequence which is employed for purification of the above form of the polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Thus, the KDI of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation. As  
25 indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein.

Amino acids in the KDI protein of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science* 244:1081-1085 (1989)). The latter  
30 procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or *in vitro* proliferative activity.

Of special interest are substitutions of charged amino acids with other charged or neutral  
35 amino acids which may produce proteins with highly desirable improved characteristics, such as less aggregation. Aggregation may not only reduce activity but also be problematic when preparing pharmaceutical formulations, because aggregates can be immunogenic (Pinckard *et al.*, *Clin. Exp. Immunol.* 2:331-340 (1967); Robbins *et al.*, *Diabetes* 36: 838-845 (1987); Cleland *et al.*, *Crit. Rev. Therapeutic Drug Carrier Systems* 10:307-377 (1993).

Replacement of amino acids can also change the selectivity of the binding of a ligand to cell surface receptors. For example, Ostade *et al.*, *Nature* 361:266-268 (1993) describes certain mutations resulting in selective binding of TNF- $\alpha$  to only one of the two known types of TNF receptors. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al.*, *J. Mol. Biol.* 224:899-904 (1992) and de Vos *et al.* *Science* 255:306-312 (1992)).

Particularly preferred substitutions for each of the KDI polypeptides described herein is the replacement of the arginine residues at position 192 with lysine (sometimes hereinafter referred to as "R192K"), and replacement of the cysteine residue at position 193 with a serine residue (sometimes hereinafter referred to as "C193S"). These substitutions can be found in a KDI polypeptide individually or they can occur in the same KDI polypeptide. Polynucleotides encoding all of the foregoing KDI polypeptides containing substitutions.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of the KDI polypeptide can be substantially purified by the one-step method described in Smith and Johnson, *Gene* 67:31-40 (1988). Polypeptides of the invention also can be purified from natural or recombinant sources using anti-KDI antibodies of the invention in methods which are well known in the art of protein purification.

The invention further provides an isolated KDI polypeptide comprising an amino acid sequence selected from the group consisting of: (a) the amino acid sequence of the full-length KDI polypeptide having the complete amino acid sequence shown in SEQ ID NO:2; (b) the amino acid sequence of the full-length KDI polypeptide having the complete amino acid sequence shown in SEQ ID NO:2 excepting the N-terminal methionine (i.e., residues 2 to 207 of SEQ ID NO:2); the amino acid sequence of the mature KDI polypeptide shown as residues 28-207 in SEQ ID NO:2; (d) the amino acid sequence shown in SEQ ID NO:2 as residues 165-183; (e) the full length KDI polypeptide encoded by the human cDNA contained in clone HKAPI15; (f) the full-length KDI polypeptide encoded by the human cDNA contained in clone HKAPI15 excepting the N-terminal methionine; and (g) the mature KDI polypeptide encoded by the human cDNA contained in clone HKAPI15.

Further polypeptides of the present invention include polypeptides which have at least 90% similarity, more preferably at least 95% similarity, and still more preferably at least 96%, 97%, 98% or 99% similarity to those described above. The polypeptides of the invention also comprise those which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptide encoded by the deposited DNA or to the polypeptide of SEQ ID NO:2, and also include portions of such polypeptides with at least 10, 20 or 30 amino acids and more preferably at least 50 amino acids.

A further embodiment of the invention relates to a peptide or polypeptide which comprises the amino acid sequence of a KDI polypeptide having an amino acid sequence which

contains at least one conservative amino acid substitution, but not more than 50 conservative amino acid substitutions, even more preferably, not more than 40 conservative amino acid substitutions, still more preferably not more than 30 conservative amino acid substitutions, and still even more preferably not more than 20 conservative amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence which comprises the amino acid sequence of a KDI polypeptide, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 conservative amino acid substitutions.

By "% similarity" for two polypeptides is intended a similarity score produced by comparing the amino acid sequences of the two polypeptides using the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711) and the default settings for determining similarity. Bestfit uses the local homology algorithm of Smith and Waterman (Advances in Applied Mathematics 2:482-489, 1981) to find the best segment of similarity between two sequences.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of an IL-20 polypeptide is intended that the amino acid sequence of the polypeptide is identical to the query sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the IL-20 polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the query sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in SEQ ID NO:2 or to the amino acid sequence encoded by deposited cDNA clone can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference

sequence are allowed. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. (1990) 6:237-245). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence

are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The polypeptide of the present invention could be used as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art.

As described in detail below, the polypeptides of the present invention can also be used to raise polyclonal and monoclonal antibodies, which are useful in assays for detecting KDI protein expression as described below or as agonists and antagonists capable of enhancing or inhibiting KDI protein function. Further, such polypeptides can be used in the yeast two-hybrid system to "capture" KDI protein binding proteins which are also candidate agonists and antagonists according to the present invention. The yeast two hybrid system is described in Fields and Song, *Nature* 340:245-246 (1989).

### ***Epitope-Bearing Portions***

In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen *et al.*, *Proc. Natl. Acad. Sci. USA* 81:3998-4002 (1983).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R. A. (1983) "Antibodies that react with predetermined sites on proteins," *Science*, 219:660-666. Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals. Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. See, for instance, Wilson *et al.*, *Cell* 37:767-778 (1984) at 777.

Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least seven, more preferably at least nine and most preferably between about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention. Non-limiting examples of antigenic polypeptides or peptides that can be used to generate KDI-specific antibodies include: a polypeptide comprising amino acid residues from



about Ser 49 to about Ser 54 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Cys 59 to about Ala 65 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Pro 78 to about Tyr 88 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about His 101 to about Gln 113 in SEQ ID NO:2; a polypeptide comprising amino acid residues Gln 120 to about Glu 123 in SEQ ID NO:2; a polypeptide comprising amino acid residues Cys 128 to about Pro 155 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Leu 160 to about Arg 168 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Asn 171 to about Asp 180 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Val 186 to about Cys 193 in SEQ ID NO:2; and a polypeptide comprising amino acid residues Phe 204 to about Lys 207 in SEQ ID NO:2. These polypeptide fragments have been determined to bear antigenic epitopes of the KDI protein by the analysis of the Jameson-Wolf antigenic index, as shown in Figure 3, above.

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. See, e.g., Houghten, R. A. (1985) "General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids." *Proc. Natl. Acad. Sci. USA* 82:5131-5135; this "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten *et al.* (1986).

Epitope-bearing peptides and polypeptides of the invention are used to induce antibodies according to methods well known in the art. See, for instance, Sutcliffe *et al.*, *supra*; Wilson *et al.*, *supra*; Chow, M. *et al.*, *Proc. Natl. Acad. Sci. USA* 82:910-914; and Bittle, F. J. *et al.*, *J. Gen. Virol.* 66:2347-2354 (1985). Immunogenic epitope-bearing peptides of the invention, i.e., those parts of a protein that elicit an antibody response when the whole protein is the immunogen, are identified according to methods known in the art. See, for instance, Geysen *et al.*, *supra*. Further still, U.S. Patent No. 5,194,392 to Geysen (1990) describes a general method of detecting or determining the sequence of monomers (amino acids or other compounds) which is a topological equivalent of the epitope (i.e., a "mimotope") which is complementary to a particular paratope (antigen binding site) of an antibody of interest. More generally, U.S. Patent No. 4,433,092 to Geysen (1989) describes a method of detecting or determining a sequence of monomers which is a topographical equivalent of a ligand which is complementary to the ligand binding site of a particular receptor of interest. Similarly, U.S. Patent No. 5,480,971 to Houghten, R. A. *et al.* (1996) on Peralkylated Oligopeptide Mixtures discloses linear C1-C7-alkyl peralkylated oligopeptides and sets and libraries of such peptides, as well as methods for using such oligopeptide sets and libraries for determining the sequence of a peralkylated oligopeptide that preferentially binds to an acceptor molecule of interest. Thus, non-peptide analogs of the epitope-bearing peptides of the invention also can be made routinely by these methods.

### ***Fusion Proteins***

As one of skill in the art will appreciate, KDI polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EP A 394,827; Traunecker et al., *Nature* 331:84-86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric KDI protein or protein fragment alone (Fountoulakis et al., *J. Biochem.* 270:3958-3964 (1995)).

### ***Antibodies***

KDI-protein specific antibodies for use in the present invention can be raised against the intact KDI protein or an antigenic polypeptide fragment thereof, which may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier.

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')<sub>2</sub> fragments) which are capable of specifically binding to KDI protein. Fab and F(ab')<sub>2</sub> fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl et al., *J. Nucl. Med.* 24:316-325 (1983)). Thus, these fragments are preferred.

The antibodies of the present invention may be prepared by any of a variety of methods. For example, cells expressing the KDI protein or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of KDI protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or KDI protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology (Köhler et al., *Nature* 256:495 (1975); Köhler et al., *Eur. J. Immunol.* 6:511 (1976); Köhler et al., *Eur. J. Immunol.* 6:292 (1976); Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., (1981) pp. 563-681). In general, such procedures involve immunizing an animal (preferably a mouse) with a KDI protein antigen or, more preferably, with a KDI protein-expressing cell. Suitable cells can be recognized by their capacity to bind anti-KDI protein antibody. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56° C), and

supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin. The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the American Type Culture Collection, Rockville, Maryland. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the KDI protein antigen.

Alternatively, additional antibodies capable of binding to the KDI protein antigen may be produced in a two-step procedure through the use of anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and that, therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, KDI-protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the KDI protein-specific antibody can be blocked by the KDI protein antigen. Such antibodies comprise anti-idiotypic antibodies to the KDI protein-specific antibody and can be used to immunize an animal to induce formation of further KDI protein-specific antibodies.

It will be appreciated that Fab and F(ab')<sub>2</sub> and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). Alternatively, KDI protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For *in vivo* use of anti-KDI in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. See, for review, Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., *Nature* 312:643 (1984); Neuberger et al., *Nature* 314:268 (1985).

## ***Immune System-Related Disorders***

### ***Treatment***

It will also be appreciated by one of ordinary skill that, since the KDI protein of the invention is a member of the interferon family, when KDI is added to cells, tissues or the body

of an individual, the protein will exert its physiological activities on its target cells of that individual. Therefore, it will be appreciated that conditions caused by a decrease in the standard or normal level of interferon activity in an individual, particularly disorders of the immune system, can be treated by administration of the KDI polypeptide. Thus, the invention also provides a method of treatment of an individual in need of an increased level of interferon activity comprising administering to such an individual a pharmaceutical composition comprising an amount of an isolated KDI polypeptide of the invention, effective to increase the interferon activity level in such an individual.

The human class I IFN receptor complex which mediates the biological activity of IFN-alpha and IFN-beta also binds IFN-omega and is expected to bind KDI. Accordingly, KDI can be used clinically for anti-viral therapy, for example, in the treatment of AIDS, viral hepatitis including chronic hepatitis B, hepatitis C, papilloma viruses, viral encephalitis, and in the prophylaxis of rhinitis and respiratory infections.

KDI is also useful in the treatment of numerous cancers (e.g., hairy cell leukemia, acute myeloid leukemia, osteosarcoma, basal cell carcinoma, glioma, renal cell carcinoma, multiple myeloma, melanoma, and Hodgkin's disease).

KDI is believed to stimulate natural killer cell activity. Accordingly, KDI may be used to treat parasitic and bacterial infection for example, for treating *Cryptosporidium parvum* infection and multidrug-resistant pulmonary tuberculosis.

KDI is also believed to be useful as an immunotherapeutic agent, more specifically as an immunosuppressive agent. For example, KDI is believed to inhibit proliferation of lymphocytes stimulated with mitogens or allogeneic cells, myeloid progenitor cells and other bone marrow cells. Accordingly, KDI is useful as a protective agent when administered prior to chemotherapy and in addition can be used to treat hyperproliferation of lymphocytes, myeloid progenitors and bone marrow stem cells, e.g., in the treatment of chronic myelogenous leukemia. KDI polypeptides can also be used in the prevention of graft vs. host rejection, or to curtail the progression of autoimmune diseases, such as arthritis, multiple sclerosis, (2) or diabetes (3). KDI is also useful in the treatment of allergies in mammals, e.g., by inhibiting the humoral response.

KDI may be used as an adjuvant or coadjuvant to enhance or simulate the immune response in cases of prophylactic or therapeutic vaccination.

Further, there is provided a method of treating infection in a patient comprising administering an effective amount of a polypeptide of the invention to a patient in need of anti-infective therapy. In a preferred embodiment the infection is of viral, bacterial, or parasitic etiology. In a particularly preferred embodiment, the infection is a viral infection.

Further, there is provided a method of treating cancer in a patient comprising administering an effective amount of a polypeptide of the invention to a patient in need of anti-cancer therapy.

Further, there is provided a method of immunotherapy in a patient comprising administering an effective amount of a polypeptide of the invention to a patient in need of immunotherapy.

### Formulations

The KDI polypeptide composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with KDI polypeptide alone), the site of delivery of the KDI polypeptide composition, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" of KDI polypeptide for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of KDI polypeptide administered parenterally per dose will be in the range of about 1  $\mu\text{g/kg/day}$  to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the KDI polypeptide is typically administered at a dose rate of about 1  $\mu\text{g/kg/hour}$  to about 50  $\mu\text{g/kg/hour}$ , either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing the KDI of the invention may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), buccally, or as an oral or nasal spray. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

The KDI polypeptide is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., *Biopolymers* 22:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (R. Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981), and R. Langer, *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., *Id.*) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988). Sustained-release KDI polypeptide compositions also include liposomally entrapped KDI polypeptide. Liposomes containing KDI polypeptide are prepared by methods known per se: DE 3,218,121; Epstein et al., *Proc. Natl.*

Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. (USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal KDI polypeptide therapy.

For parenteral administration, in one embodiment, the KDI polypeptide is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting the KDI polypeptide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The KDI polypeptide is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of KDI polypeptide salts.

KDI polypeptide to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic KDI polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

KDI polypeptide ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous KDI polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized KDI polypeptide using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

#### ***Agonists and Antagonists - Assays and Molecules***

The invention also provides a method of screening compounds to identify those which enhance or block the action of KDI on cells, such as its interaction with KDI-binding molecules such as receptor molecules. An agonist is a compound which increases the natural biological functions of KDI or which functions in a manner similar to KDI, while antagonists decrease or eliminate such functions.

In another aspect of this embodiment the invention provides a method for identifying a receptor protein or other ligand-binding protein which binds specifically to a KDI polypeptide. For example, a cellular compartment, such as a membrane or a preparation thereof, may be prepared from a cell that expresses a molecule that binds KDI. The preparation is incubated with labeled KDI. KDI and complexes of KDI bound to the receptor or other binding protein are isolated and characterized according to routine methods known in the art. Alternatively, the KDI polypeptide may be bound to a solid support so that binding molecules solubilized from cells are bound to the column and then eluted and characterized according to routine methods.

In the assay of the invention for agonists or antagonists, a cellular compartment, such as a membrane or a preparation thereof, may be prepared from a cell that expresses a molecule that binds KDI, such as a molecule of a signaling or regulatory pathway modulated by KDI. The preparation is incubated with labeled KDI in the absence or the presence of a candidate molecule which may be a KDI agonist or antagonist. The ability of the candidate molecule to bind the binding molecule is reflected in decreased binding of the labeled ligand. Molecules which bind gratuitously, i.e., without inducing the effects of KDI on binding the KDI binding molecule, are most likely to be good antagonists. Molecules that bind well and elicit effects that are the same as or closely related to KDI are agonists.

KDI-like effects of potential agonists and antagonists may be measured, for instance, by determining activity of a second messenger system following interaction of the candidate molecule with a cell or appropriate cell preparation, and comparing the effect with that of KDI or molecules that elicit the same effects as KDI. Second messenger systems that may be useful in this regard include but are not limited to AMP guanylate cyclase, ion channel or phosphoinositide hydrolysis second messenger systems.

Another example of an assay for KDI antagonists is a competitive assay that combines KDI and a potential antagonist with membrane-bound KDI receptor molecules or recombinant KDI receptor molecules under appropriate conditions for a competitive inhibition assay. KDI can be labeled, such as by radioactivity, such that the number of KDI molecules bound to a receptor molecule can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to a polypeptide of the invention and thereby inhibit or extinguish its activity. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a receptor molecule, without inducing KDI-induced activities, thereby preventing the action of KDI by excluding KDI from binding.

Other potential antagonists include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, *J. Neurochem.* 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression." CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee *et al.*, *Nucleic Acids Research* 6: 3073 (1979); Cooney *et al.*, *Science* 241: 456 (1988); and Dervan *et al.*, *Science* 251: 1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA. For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of KDI. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into KDI polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of KDI protein.

The agonists and antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described above.

The antagonists may be employed for instance to inhibit interferon activity, for example, following chemotherapy to stimulate proliferation of bone marrow and haematopoietic



progenitor cells. Any of the above antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as hereinafter described.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

### ***Examples***

#### ***Example 1: Cloning and Expression of KDI in E. coli***

The novel pHE4 series of bacterial expression vectors, in particular, the pHE4a vector is used for bacterial expression in this example. (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311). pHE4-5/KDI vector plasmid DNA contains the KDI coding polynucleotide shown in Figure 1 inserted between unique restriction enzyme sites *NdeI* and *Asp718*. The construct was deposited with the ATCC on February 25, 1998 and given Accession No. 209645, as a convenience to those of skill in the art.

The pHE4a bacterial expression vector includes a neomycin phosphotransferase gene for selection, an *E. coli* origin of replication, a T5 phage promoter sequence, two *lac* operator sequences, a Shine-Delgarno sequence, and the lactose operon repressor gene (*lacIq*). These elements are arranged such that an inserted DNA fragment encoding a polypeptide expresses that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the amino terminus of that polypeptide.

The DNA sequence encoding the mature KDI protein is amplified using PCR oligonucleotide primers which anneal to the amino terminal sequences of the desired portion of the KDI protein and to sequences in the deposited construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pHE4a vector are added to the 5' and 3' primer sequences, respectively.

For cloning the KDI protein coding region, the 5' primer has the sequence 5' GGCCGCATATGCTGGACTGTAACCTTACTG 3' (SEQ ID NO:16) containing the underlined *NdeI* restriction site. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a DNA segment encoding any desired portion of the complete KDI protein. The 3' primer has the sequence 5' GGCCGCGGTACCTTATTTCTCCTGAATAGAGC 3' (SEQ ID NO:17) containing the underlined *Asp718* restriction site.

The amplified KDI DNA fragment is digested with *NdeI* and *Asp718* and it and the linearized plasmid are then ligated together. Insertion of the KDI DNA into the restricted pHE4a vector places the KDI protein coding region downstream from the IPTG-inducible promoter and in-frame with an initiating AUG and the six histidine codons.

The ligation mixture is transformed into competent *E. coli* cells using standard procedures such as those described by Sambrook and colleagues (*Molecular Cloning: a Laboratory Manual, 2nd Ed.*; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989)). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac repressor and confers kanamycin resistance ("Kanr"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing KDI protein, is available commercially (QIAGEN, Inc., *supra*). Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing.

Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 µg/ml) and kanamycin (25 µg/ml). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:25 to 1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. Isopropyl-β-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from the lac repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

The cells are then stirred for 3-4 hours at 4°C in 6M guanidine-HCl, pH 8. The cell debris is removed by centrifugation, and the supernatant containing the KDI polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist, 1995, QIAGEN, Inc., *supra*). Briefly the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the KDI is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins can be eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50

mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4° C or frozen at -80° C.

The following alternative method may be used to purify KDI expressed in *E coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10°C.

Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10°C and the cells are harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells were then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 x g for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 x g centrifugation for 15 min., the pellet is discarded and the KDI polypeptide-containing supernatant is incubated at 4°C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 x g) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4°C without mixing for 12 hours prior to further purification steps.

To clarify the refolded KDI polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 µm membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the KDI polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant  $A_{280}$  monitoring of the effluent. Fractions containing the KDI polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant KDI polypeptide exhibits greater than 95% purity after the above refolding and purification steps. No major contaminant bands are observed from Commassie blue stained 16% SDS-PAGE gel when 5  $\mu$ g of purified protein is loaded. The purified protein is also tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

The following alternative method may be used to purify KDI expressed in *E. coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10°C.

Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10°C and the cells are harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells were then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 x g for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 x g centrifugation for 15 min., the pellet is discarded and the KDI polypeptide-containing supernatant is incubated at 4°C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 x g) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring.

The refolded diluted protein solution is kept at 4°C without mixing for 12 hours prior to further purification steps.

To clarify the refolded KDI polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 µm membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the KDI polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant  $A_{280}$  monitoring of the effluent. Fractions containing the KDI polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant KDI polypeptide exhibits greater than 95% purity after the above refolding and purification steps. No major contaminant bands are observed from Commassie blue stained 16% SDS-PAGE gel when 5 µg of purified protein is loaded. The purified protein is also tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

Multiple KDI expression constructs have been generated by the inventors herein to facilitate the production of KDI polypeptides of several sizes and in several systems. The E. coli based constructs are as follows: (1) pQE9:KDI.S27-K207 (expresses amino acids 27-207 of SEQ ID NO:2); (2) pHE4:KDI.S27-K207 (expresses amino acids 27-207 of SEQ ID NO:2); (3) pHE4:KDI.A23-K207 (expresses amino acids 23-207 of SEQ ID NO:2); (4) pHE4:KDI.G24-K207 (expresses amino acids 24-207 of SEQ ID NO:2); and (5) pHE4:KDI.C30-K207 (expresses amino acids 30-207 of SEQ ID NO:2).

### ***Example 2: Cloning and Expression of KDI protein in a Baculovirus Expression System***

In this illustrative example, the plasmid shuttle vector pA2 GP is used to insert the cloned DNA encoding KDI, into a baculovirus to express the KDI protein, using a baculovirus leader and standard methods as described in Summers et al., *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by the secretory signal peptide (leader) of the baculovirus gp67 protein and convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that expresses the cloned polynucleotide.

Many other baculovirus vectors could be used in place of the vector above, such as pAc373, pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology* 170:31-39 (19989).

The cDNA sequence encoding the mature KDI protein in the deposited clone, lacking the AUG initiation codon and the naturally associated leader sequence shown in SEQ ID NO:2, is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5' GGCCGGGATCCGCCATCATGAGCACCAAACCTGATATG 3' (SEQ ID NO:18) containing the underlined BamHI restriction enzyme site. The 3' primer has the sequence 5' GGCCGCGGTACCTTATTCCTCCTGAATAGAGC 3' (SEQ ID NO:19) containing the underlined Asp718 restriction site.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with BamHI and Asp718 and again is purified on a 1% agarose gel.

The plasmid is digested with the restriction enzymes BamHI and Asp718 and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures

known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

Fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Statagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria are identified that contain the plasmid with the human KDI gene by digesting DNA from individual colonies using BamHI and Asp718 and then analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pA2GPKDI.

Five µg of the plasmid pA2GPKDI is co-transfected with 1.0 µg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., *Proc. Natl. Acad. Sci. USA* 84: 7413-7417 (1987). One µg of BaculoGold™ virus DNA and 5 µg of the plasmid pA2GPKDI are mixed in a sterile well of a microtiter plate containing 50 µl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 µl Lipofectin plus 90 µl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27° C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27° C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10). After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 µl of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4° C. The recombinant virus is called V-KDI.

To verify the expression of the KDI gene Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus V-KDI at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus

methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5  $\mu$ Ci of  $^{35}$ S-methionine and 5  $\mu$ Ci  $^{35}$ S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the of the KDI protein.

Other baculovirus expression constructs were constructed as follows: (1) pA2:KDI (which expresses residues 1-207 of SEQ ID NO:2); (2) pA2.KDI.M7-K207 (which expresses residues 7-207 of SEQ ID NO:2); (3) pA2gp.KDI.L28-K207 (which expresses residues 28-207 of SEQ ID NO:2); (4) pA2gp.KDI.C30-K207 (which expresses residues 30-207 of SEQ ID NO:2); and (5) pA2.KDI.M1-R192 (which expresses residues 1 to 192 of SEQ ID NO:2).

### ***Example 3: Cloning and Expression of KDI in Mammalian Cells***

A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLV I, HIV I and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include, human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., *Biochem J.* 227:277-279 (1991); Bebbington et al., *Bio/Technology* 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are



selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen *et al.*, *Molecular and Cellular Biology*, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart *et al.*, *Cell* 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

### ***Cloning and Expression in CHO Cells***

The vector pC4-Sig is used for the expression of KDI polypeptide. Plasmid pC4-Sig is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). It contains coding region for the secretory leader sequence from chemokine beta-8 (see US95/09508) upstream from the multiple cloning site and is designed to be inframe with inserted heterologous DNA. The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., Alt, F. W., Kellems, R. M., Bertino, J. R., and Schimke, R. T., 1978, *J. Biol. Chem.* 253:1357-1370, Hamlin, J. L. and Ma, C. 1990, *Biochem. et Biophys. Acta*, 1097:107-143, Page, M. J. and Sydenham, M. A. 1991, *Biotechnology* 9:64-68). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach may be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained which contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rouse Sarcoma Virus (Cullen, *et al.*, *Molecular and Cellular Biology*, March 1985:438-447) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart *et al.*, *Cell* 41:521-530 (1985)). Downstream of the promoter are the following single restriction enzyme cleavage sites that allow the integration of the genes: BamHI, Xba I, and Asp718. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human  $\beta$ -actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses.

e.g., HIV and HTLVI. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the KDI polypeptide in a regulated way in mammalian cells (Gossen, M., & Bujard, H. 1992, *Proc. Natl. Acad. Sci. USA* 89:5547-5551). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with the restriction enzymes BamHI and Asp718 and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

The DNA sequence encoding the KDI polypeptide is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the desired portion of the gene. The 5' primer containing the underlined BamHI site, a Kozak sequence, and an AUG start codon, has the following sequence:

5' GGCCGGGATCCGCCATCATGAGCACCAAACCTGATATG 3' (SEQ ID NO:18). The 3' primer, containing the underlined Asp718 restriction site has the following sequence: 5' GGCCGCGGTACCTTATTCCTCCTGAATAGAGC 3' (SEQ ID NO:19).

The amplified fragment is digested with the endonucleases BamHI and Asp718 and then purified again on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene are used for transfection. Five µg of the expression plasmid pC4 is cotransfected with 0.5 µg of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 µM, 2 µM, 5 µM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 µM. Expression of the desired

gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

Other mammalian expression vectors constructed are as follows: (1) pC4:KDI (which expresses residues 1-207 of SEQ ID NO:2); (2) pC4sp:KDI.C30-K207 (which expresses a heterologous signal peptide (the chemokine beta-8 (MPIF-1) signal peptide) followed by amino acid residues 30-207 of KDI; and (3) pC4sp:KDI.L28-K207 (which expresses a heterologous signal peptide (the chemokine beta-8 (MPIF-1) signal peptide) followed by amino acid residues 28-207 of KDI.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of all publications (including patents, patent applications, journal articles, laboratory manuals, books, or other documents) cited herein are hereby incorporated by reference.

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>7</u> , line <u>10</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit  December 1, 1998	Accession Number  203500
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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***What Is Claimed Is:***

1. An isolated polynucleotide comprising a nucleic acid sequence at least 95% identical to a member selected from the group consisting of:
  - (a) a nucleotide sequence encoding the polypeptide shown as residues 1 to 207 of SEQ ID NO:2;
  - (b) a nucleotide sequence encoding the polypeptide shown as residues 2 to 207 of SEQ ID NO:2;
  - (c) a nucleotide sequence encoding the polypeptide shown as residues 28 to 207 of SEQ ID NO:2;
  - (d) a nucleotide sequence encoding the polypeptide shown as residues 30 to 207 of SEQ ID NO:2;
  - (e) a nucleotide sequence encoding the polypeptide shown as residues 165 to 183 of SEQ ID NO:2;
  - (f) a nucleotide sequence encoding the complete polypeptide encoded by the human cDNA contained in clone HKAPI15;
  - (g) a nucleotide sequence encoding the complete polypeptide encoded by the human cDNA contained in clone HKAPI15 excepting the N-terminal methionine;
  - (h) a nucleotide sequence encoding the mature polypeptide encoded by the human cDNA contained in clone HKAPI15; and
  - (i) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), or (h) above.
2. An isolated polynucleotide comprising a nucleic acid sequence selected from the group consisting of:
  - (a) a nucleotide sequence which encodes a biologically active fragment of the polypeptide shown as residues 1 to 207 of SEQ ID NO:2; and
  - (b) a nucleotide sequence complementary to the nucleotide sequence of (a).
3. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figure 1 (SEQ ID NO:1) encoding the KDI polypeptide having the amino acid sequence in positions 165 to 183 of SEQ ID NO:2.
4. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figure 1 (SEQ ID NO:1) encoding the KDI polypeptide having the amino acid sequence in positions 28 to 207 of SEQ ID NO:2.
5. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

- (a) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of residues n-207 of SEQ ID NO:2, where n is an integer in the range of 1-59;
- (b) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of residues 1-m of SEQ ID NO:2, where m is an integer in the range of 183-207;
- (c) a nucleotide sequence encoding a polypeptide having the amino acid sequence consisting of residues n-m of SEQ ID NO:2, where n and m are integers as defined respectively in (a) and (b) above;
- (d) a nucleotide sequence encoding the polypeptide encoded by the human cDNA in clone HKAPI15 wherein said polypeptide is lacking between 1 and 58 amino acids from its N-terminus;
- (e) a nucleotide sequence encoding the polypeptide encoded by the human cDNA in clone HKAPI15 wherein said polypeptide is lacking between 1 and 23 amino acids from its C-terminus; and
- (f) a nucleotide sequence encoding the polypeptide encoded by the human cDNA in clone HKAPI15 wherein said polypeptide has any combination of N-terminal and C-terminal deletions described in (d) and (e), above.

6. An isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c), (d), (e), (f), (g) or (h) of claim 1 wherein said polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

7. An isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a KDI polypeptide having an amino acid sequence in (a), (b), (c), (d), (e), (f) or (g) of claim 1.

8. The isolated nucleic acid molecule of claim 7 comprising a nucleic acid sequence which encodes an epitope-bearing portion of a KDI polypeptide selected from the group consisting of: a polypeptide comprising amino acid residues from about Ser 49 to about Ser 54 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Cys 59 to about Ala 65 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Pro 78 to about Tyr 88 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about His 101 to about Gln 113 in SEQ ID NO:2; a polypeptide comprising amino acid residues Gln 120 to about Glu 123 in SEQ ID NO:2; a polypeptide comprising amino acid residues Cys 128 to about Pro 155 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Leu 160 to about Arg 168 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Asn 171 to about Asp 180 in SEQ ID NO:2; a polypeptide comprising amino acid

residues from about Val 186 to about Cys 193 in SEQ ID NO:2; and a polypeptide comprising amino acid residues Phe 204 to about Lys 207 in SEQ ID NO:2.

9. A method for making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim 1 into a vector.

10. A recombinant vector produced by the method of claim 9.

11. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 10 into a host cell.

12. A recombinant host cell produced by the method of claim 11.

13. A recombinant method for producing a KDI polypeptide, comprising culturing the recombinant host cell of claim 12 under conditions such that said polypeptide is expressed and recovering said polypeptide.

14. An isolated KDI polypeptide comprising an amino acid sequence at least 95% identical to a member selected from the group consisting of:

- (a) the polypeptide shown as residues 1 to 207 of SEQ ID NO:2;
- (b) the polypeptide shown as residues 2 to 207 of SEQ ID NO:2;
- (c) the polypeptide shown as residues 28 to 207 of SEQ ID NO:2;
- (d) the polypeptide shown as residues 165 to 183 of SEQ ID NO:2;
- (e) the complete polypeptide encoded by the human cDNA contained in clone HKAPI15;
- (f) the complete polypeptide encoded by the human cDNA contained in clone HKAPI15 excepting the N-terminal methionine;
- (g) the mature polypeptide encoded by the human cDNA contained in clone HKAPI15.

15. An isolated polypeptide comprising an epitope-bearing portion of the KDI protein, wherein said portion is selected from the group consisting of: a polypeptide comprising amino acid residues from about Ser 49 to about Ser 54 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Cys 59 to about Ala 65 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Pro 78 to about Tyr 88 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about His 101 to about Gln 113 in SEQ ID NO:2; a polypeptide comprising amino acid residues Gln 120 to about Glu 123 in SEQ ID NO:2; a polypeptide comprising amino acid residues Cys 128 to about Pro 155 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Leu 160 to about Arg 168 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Asn 171 to about Asp

180 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Val 186 to about Cys 193 in SEQ ID NO:2; and a polypeptide comprising amino acid residues Phe 204 to about Lys 207 in SEQ ID NO:2.

16. An isolated antibody that binds specifically to a KDI polypeptide of claim 14.
17. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence which is at least 95% identical at least 50 contiguous nucleotides from SEQ ID NO:1.
18. An isolated polypeptide comprising an amino acid sequence of a biologically active fragment of the polypeptide shown as residues 1 to 207 of SEQ ID NO:2.
19. A pharmaceutical composition comprising a polypeptide of claim 14 in a pharmaceutically acceptable carrier.
20. A method of treating viral infection in a patient comprising administering to the patient the composition of claim 19.



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## Keratinocyte Derived Interferon

```

1 CCA CGC GTC CGG GAT TTT TTA GCT TGC AAA AAA AAT GAG CAC CAA ACC TGA TAT GAT TCA 60
1 M S T K P D M L Q 9

61 AAA GTG TTT GTG GCT TGA GAT CCT TAT GGG TAT ATT CAT TGC TGG CAC CCT ATC CCT GGA 120
9 K C L W L E I L M G I F I A G T L S L D 29

121 CTG TAA CTT ACT GAA CGT TCA CCT GAG AAG AGT CAC CTG GCA AAA TCT GAG ACA TCT GAG 180
29 C N L L N V H L R R V T W Q N L R H L S 49

181 TAG TAT GAG CAA TTC ATT TCC TGT AGA ATG TCT ACG AGA AAA CAT AGC TTT TGA GTT GCC 240
49 S M S N S F P V E C L R E N I A F E L P 69

241 CCA AGA GTT TCT GCA ATA CAC CCA ACC TAT GAA GAG GGA CAT CAA GAA GGC CTT CTA TGA 300
69 Q E F L Q Y T Q P M K R D I K K A F Y E 89

301 AAT GTC CCT ACA GGC CTT CAA CAT CTT CAG CCA ACA CAC CTT CAA ATA TTG GAA AGA GAG 360
89 M S L Q A F N I F S Q H T F K Y W K E R 109

361 ACA CCT CAA ACA AAT CCA AAT AGG ACT TGA TCA GCA AGC AGA GTA CCT GAA CCA ATG CTT 420
109 H L K Q I Q I G L D Q Q A E Y L N Q C L 129

421 GGA GGA AGA CGA GAA TGA AAA TGA AGA CAT GAA AGA AAT GAA AGA GAA TGA GAT GAA ACC 480
129 E E D E N E N E D M K E M K E N E M K P 149

481 CTC AGA AGC CAG GGT CCC CCA GCT GAG CAG CCT GGA ACT GAG GAG ATA TTT CCA CAG GAT 540
149 S E A R V P Q L S S L E L R R Y F H R I 169

541 AGA CAA TTT CCT GAA AGA AAA GAA ATA CAG TGA CTG TGC CTG GGA GAT TGT CCG AGT GGA 600
169 D N F L K E K K Y S D C A W E I V R V E 189

601 AAT CAG AAG ATG TTT GTA TTA CTT TTA CAA ATT TAC AGC TCT ATT CAG GAG GAA ATA AGA 660
189 I R R C L Y Y F Y K F T A L F R R K * 207

661 ATC ATC TAC CTT CAA GCA AGA ATT AAC AGA GAT TGT GGC TAC GCA AAT GCA CCA AAA AAG 720
721 GGT GAA ATA TAT CTG AAA TGT ACC TGG TTC TGC CCT TGG AAG CCA CTT CCT GCT CAT GCC 780
781 ACT AAC AGC ATG CTG CCA AAC TGT TCA GAT TCA AGA TTA TTC CAA GCG CAG GGC CCA AAT 840
841 GTT ATA GCC AAA GAA AGT CTT ATG ATA AAA GTG AGG CAA ATT TCA GCC AAG AAG TTA GAA 900
901 GAG ATG TTT AAA AGA ACA AGA ACA AAT TGT GGA TCA TGG TAT ATG CAG GCT ATC AGC AGA 960
961 AGG ATC AGA CAA TAA AAT GAG TTA GTG CAA ACC ATT TAG TAA AAA TAA CTA TCA GCA GAG 1020
1021 TTG TTC CAG ATT AAA AAT AGT ACT ACA AGC TTG TAA AGG AGT TAG GAC ATG CAA GCT ACT 1080
1081 GAG CAT AAA ATA TAT ACT TGC TAT TTT TCA TGA CTT TCT CTA ATA AAG TCT TTG ACT GTT 1140
1141 CTC TCT AAT AAA AAA AAA AAA AAA AAA 1170

```

FIG. 1

27

HKAPI15	10	KCLWLEILMGIFIAG...TSLDCNLLNVH...LRRVTWQNLRLHLSMSNS	54
		.. :::  :::: .   ::  .    .   :  :::..	
INF-omega	1	MALLFPLLAALVMTSYSPVGSGLGCDLPQNHGLLSRNTLVLLHQMRRIS..	48
HKAPI15	55	FPVECLRENIAFELPQEFLOQTQPMKRDIKKAFYEMSLOAFNIF.SQHTF	103
		.   :: .   :  :: .     .....     .   ::.	
INF-omega	49	.PFLCLKDRRDFRFPQEMVKSQQLQKAHVMSVLHEMLQQIFSLFHTERS	97
HKAPI15	104	KYWKERHLKQIQIGLDQQAAYLNQCLEEDENENEDMKEMKENEMKPSEAR	153
		.  .  ::: .    :.   :   : .  .  . ....	
INF-omega	98	AAWNMTLLDQLHTELHQQLQHLETCLLQVVGESESAGAISS.....	138
HKAPI15	154	VPQLSSLELRRYFHRIDNFLKEKKYSDCAWEIVRVEIRRCLYYFYKFTAL	203
		.. .     :   :       ::  ::  ::: :.. :.	
INF-omega	139	....PALTLRRYFQIRVYLKEKKYSDCAWEVVRMEIMKSLFLSTNMQER	184
HKAPI15	204	FRRK	207
		: .	
INF-omega	185	LRSK	188

FIG. 2

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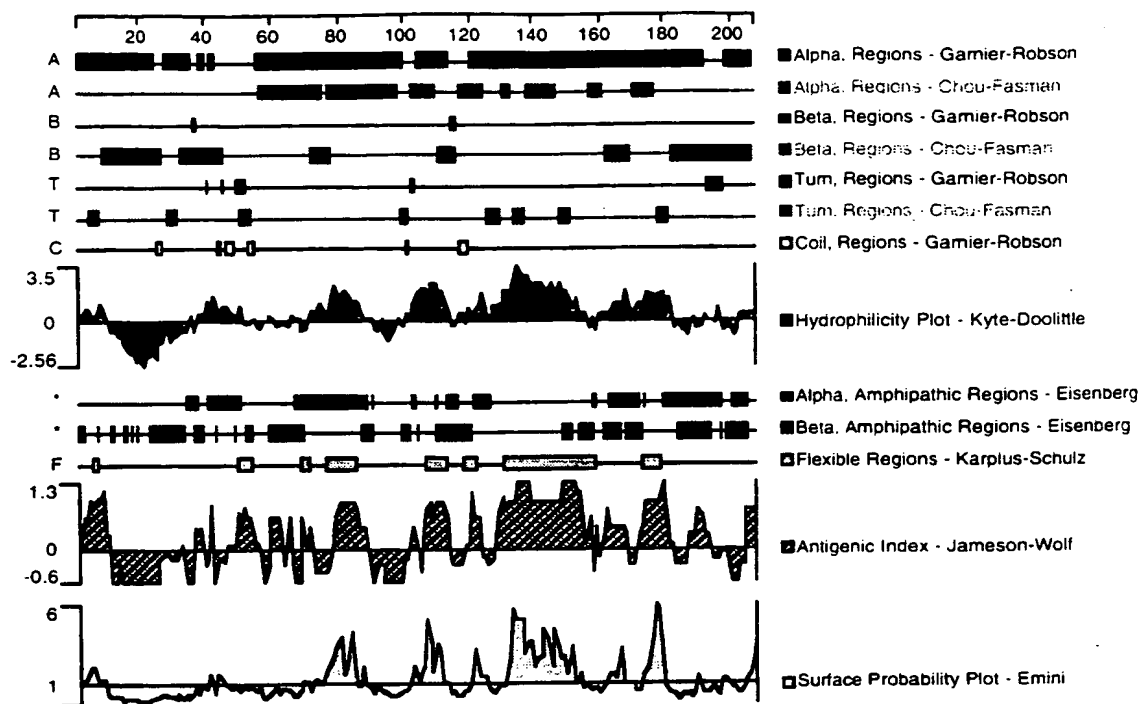


FIG. 3

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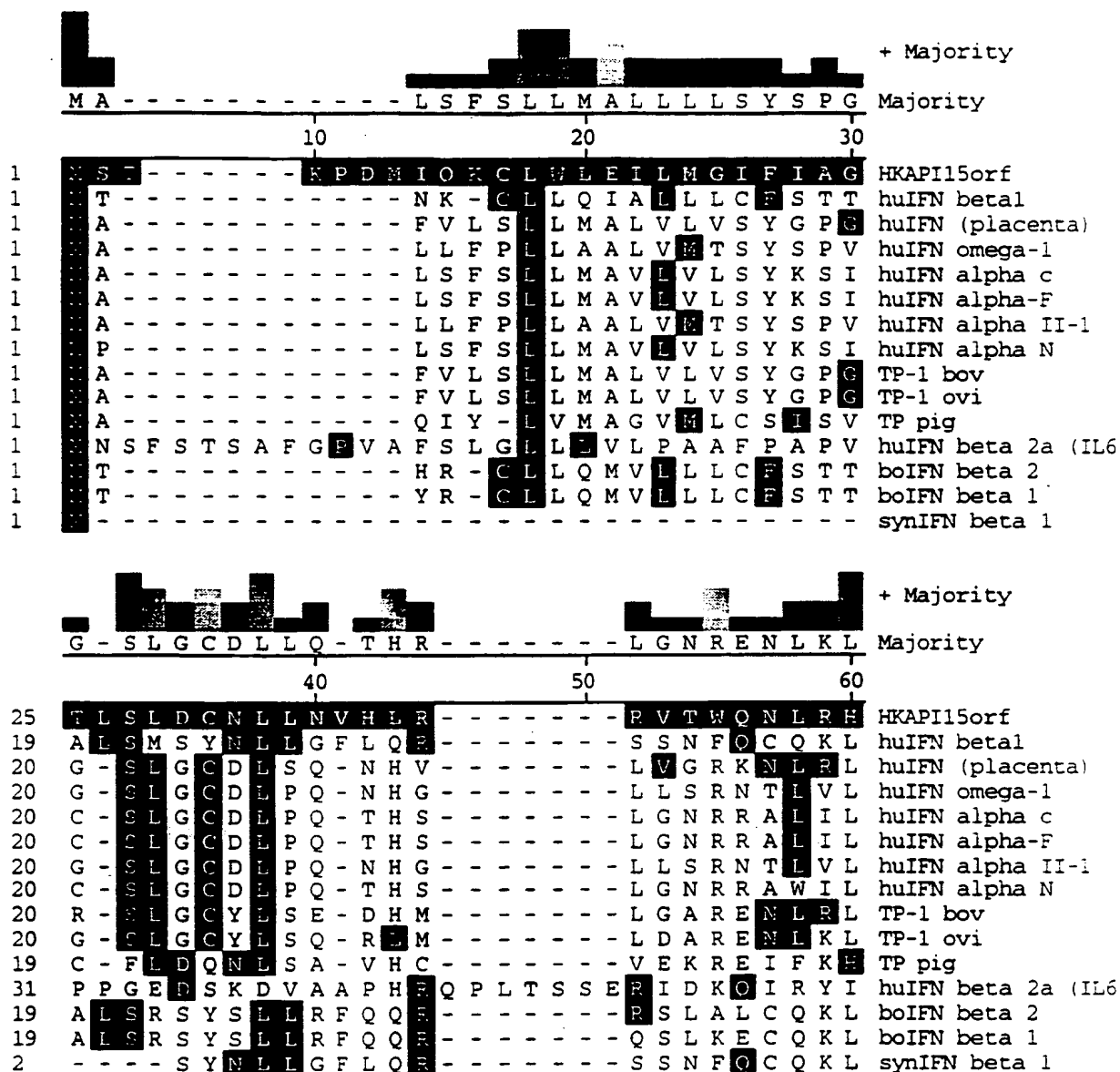


FIG. 4A

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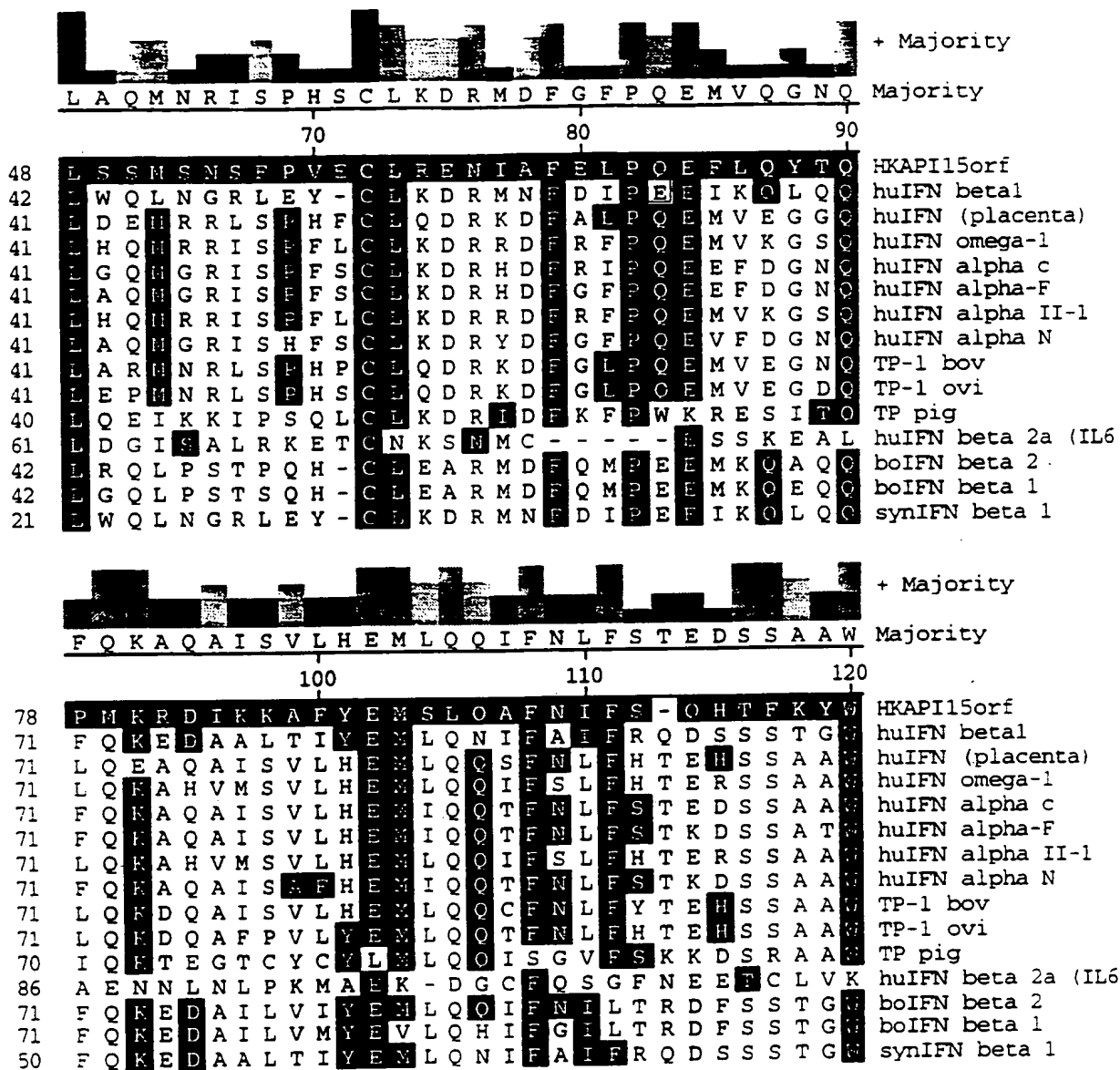


FIG. 4B

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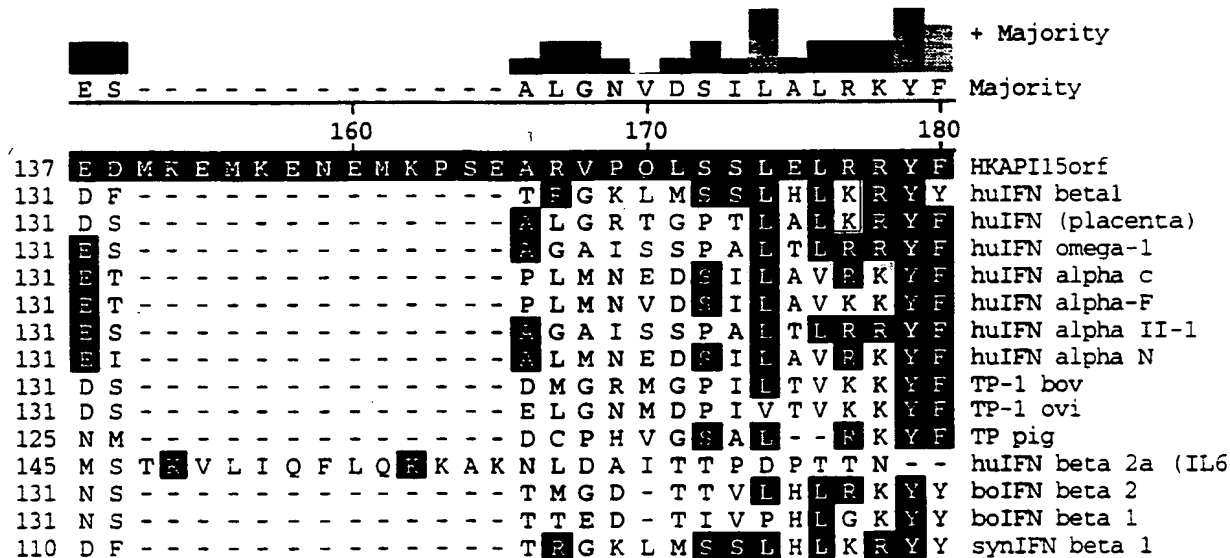
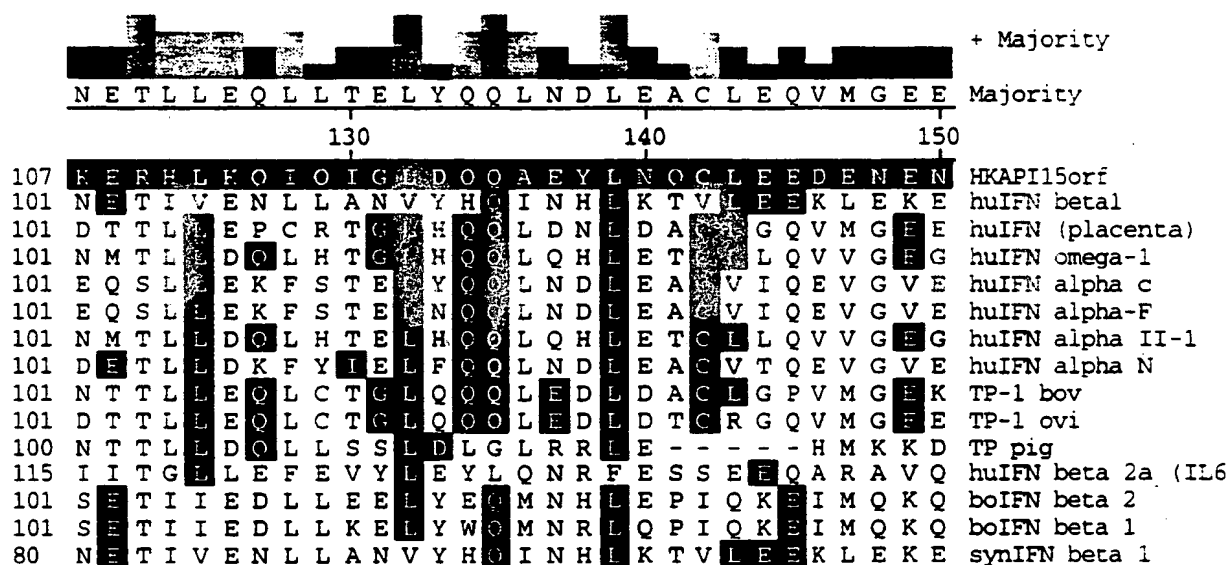


FIG. 4C

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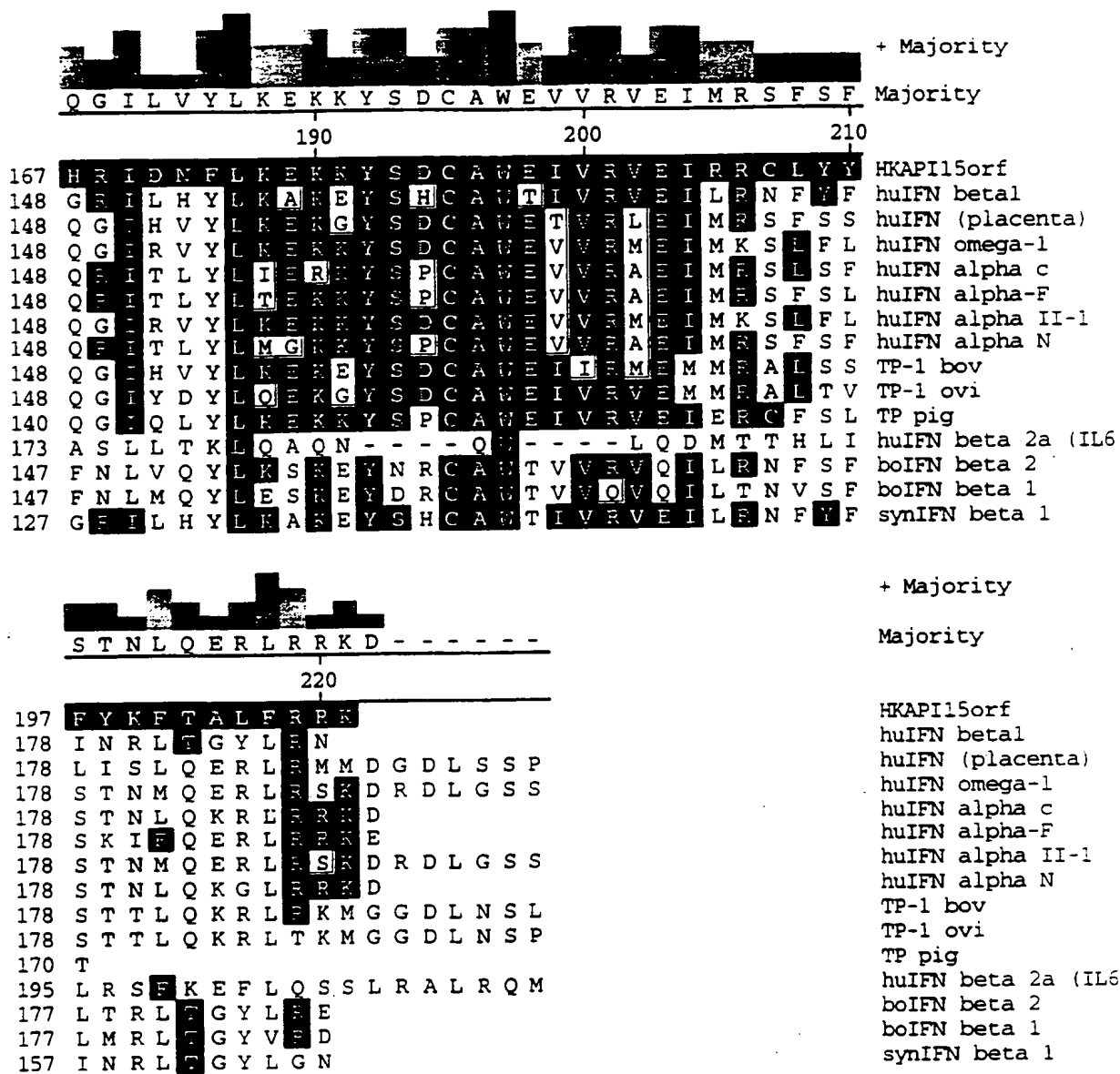


FIG. 4D

## SEQUENCE LISTING

&lt;110&gt; Human Genome Sciences, Inc., et al.

&lt;120&gt; Keratinocyte Derived Interferon

&lt;130&gt; PF482

&lt;140&gt; Unassigned

&lt;141&gt; 1999-07-21

&lt;150&gt; 60/093,643

&lt;151&gt; 1998-07-21

&lt;160&gt; 21

&lt;170&gt; PatentIn Ver. 2.0

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att caa aag tgt ttg tgg ctt gag atc ctt atg ggt ata ttc att gct 103  
Ile Gln Lys Cys Leu Trp Leu Glu Ile Leu Met Gly Ile Phe Ile Ala  
10 15 20

ggc acc cta tcc ctg gac tgt aac tta ctg aac gtt cac ctg aga aga 151  
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Val Thr Trp Gln Asn Leu Arg His Leu Ser Ser Met Ser Asn Ser Phe  
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cct gta gaa tgt cta cga gaa aac ata gct ttt gag ttg ccc caa gag 247  
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60 65 70

ttt ctg caa tac acc caa cct atg aag agg gac atc aag aag gcc ttc 295  
Phe Leu Gln Tyr Thr Gln Pro Met Lys Arg Asp Ile Lys Lys Ala Phe  
75 80 85

tat gaa atg tcc cta cag gcc ttc aac atc ttc agc caa cac acc ttc 343  
Tyr Glu Met Ser Leu Gln Ala Phe Asn Ile Phe Ser Gln His Thr Phe  
90 95 100

aaa tat tgg aaa gag aga cac ctc aaa caa atc caa ata gga ctt gat 391  
Lys Tyr Trp Lys Glu Arg His Leu Lys Gln Ile Gln Ile Gly Leu Asp  
105 110 115

cag caa gca gag tac ctg aac caa tgc ttg gag gaa gac gag aat gaa 439



Gln Gln Ala Glu Tyr Leu Asn Gln Cys Leu Glu Glu Asp Glu Asn Glu  
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&lt;211&gt; 207

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

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 20 25 30

Leu Asn Val His Leu Arg Arg Val Thr Trp Gln Asn Leu Arg His Leu  
 35 40 45

Ser Ser Met Ser Asn Ser Phe Pro Val Glu Cys Leu Arg Glu Asn Ile  
 50 55 60

Ala Phe Glu Leu Pro Gln Glu Phe Leu Gln Tyr Thr Gln Pro Met Lys

65		70		75		80									
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Ile	Phe	Ser	Gln	His	Thr	Phe	Lys	Tyr	Trp	Lys	Glu	Arg	His	Leu	Lys
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Leu	Glu	Glu	Asp	Glu	Asn	Glu	Asn	Glu	Asp	Met	Lys	Glu	Met	Lys	Glu
	130					135					140				
Asn	Glu	Met	Lys	Pro	Ser	Glu	Ala	Arg	Val	Pro	Gln	Leu	Ser	Ser	Leu
145					150					155					160
Glu	Leu	Arg	Arg	Tyr	Phe	His	Arg	Ile	Asp	Asn	Phe	Leu	Lys	Glu	Lys
				165					170					175	
Lys	Tyr	Ser	Asp	Cys	Ala	Trp	Glu	Ile	Val	Arg	Val	Glu	Ile	Arg	Arg
			180					185					190		
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&lt;211&gt; 238

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			20					25					30		
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Pro	Phe	Leu	Cys	Leu	Lys	Asp	Arg	Arg	Asp	Phe	Arg	Phe	Pro	Gln	Glu
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Met	Val	Lys	Gly	Ser	Gln	Leu	Gln	Lys	Ala	His	Val	Met	Ser	Val	Leu
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His	Glu	Met	Leu	Gln	Gln	Ile	Phe	Ser	Leu	Phe	His	Thr	Glu	Arg	Ser
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			100						105					110	
His	Gln	Gln	Leu	Gln	His	Leu	Glu	Thr	Cys	Leu	Leu	Gln	Val	Val	Gly
		115					120					125			
Glu	Gly	Glu	Ser	Ala	Gly	Ala	Ile	Ser	Ser	Val	Pro	Gln	Leu	Ser	Ser
	130					135					140				
Leu	Glu	Leu	Arg	Arg	Tyr	Phe	His	Arg	Ile	Asp	Asn	Phe	Leu	Lys	Glu

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    165                                      170                                      175  
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    180                                      185                                      190  
 Leu Arg Arg Tyr Phe Gln Gly Ile Arg Val Tyr Leu Lys Glu Lys Lys  
    195                                      200                                      205  
 Tyr Ser Asp Cys Ala Trp Glu Val Val Arg Met Glu Ile Met Lys Ser  
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 Ser Ser Asn Phe Gln Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg  
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 Leu Glu Tyr Cys Leu Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu  
   50  55  60  
 Ile Lys Gln Leu Gln Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile  
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 Tyr Glu Met Leu Gln Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser  
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 Tyr His Gln Ile Asn His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu  
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 Lys Glu Asp Phe Thr Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys  
    130   135   140  
 Arg Tyr Tyr Gly Arg Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser  
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 His Cys Ala Trp Thr Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr  
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 Pro His Phe Cys Leu Gln Asp Arg Lys Asp Phe Ala Leu Pro Gln Glu  
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 Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile Ser Val Leu  
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 His Gln Gln Leu Asp Asn Leu Asp Ala Cys Leu Gly Gln Val Met Gly  
           115                          120                          125  
 Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Leu Ala Leu Lys Arg  
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 Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys Gly Tyr Ser Asp  
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 Cys Ala Trp Glu Thr Val Arg Leu Glu Ile Met Arg Ser Phe Ser Ser  
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 Ser Pro

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                           20                          25                          30  
 Leu Ser Arg Asn Thr Leu Val Leu Leu His Gln Met Arg Arg Ile Ser  
           35                          40                          45

Pro Phe Leu Cys Leu Lys Asp Arg Arg Asp Phe Arg Phe Pro Gln Glu  
 50 55 60  
 Met Val Lys Gly Ser Gln Leu Gln Lys Ala His Val Met Ser Val Leu  
 65 70 75 80  
 His Glu Met Leu Gln Gln Ile Phe Ser Leu Phe His Thr Glu Arg Ser  
 85 90 95  
 Ser Ala Ala Trp Asn Met Thr Leu Leu Asp Gln Leu His Thr Glu Leu  
 100 105 110  
 His Gln Gln Leu Gln His Leu Glu Thr Cys Leu Leu Gln Val Val Gly  
 115 120 125  
 Glu Gly Glu Ser Ala Gly Ala Ile Ser Ser Val Pro Gln Leu Ser Ser  
 130 135 140  
 Leu Glu Leu Arg Arg Tyr Phe His Arg Ile Asp Asn Phe Leu Lys Glu  
 145 150 155 160  
 Lys Lys Tyr Ser Asp Cys Ala Trp Glu Ile Val Arg Val Glu Ile Arg  
 165 170 175  
 Arg Cys Leu Tyr Tyr Phe Tyr Lys Phe Thr Ala Leu Pro Ala Leu Thr  
 180 185 190  
 Leu Arg Arg Tyr Phe Gln Gly Ile Arg Val Tyr Leu Lys Glu Lys Lys  
 195 200 205  
 Tyr Ser Asp Cys Ala Trp Glu Val Val Arg Met Glu Ile Met Lys Ser  
 210 215 220  
 Leu Phe Leu Ser Thr Asn Met Gln Glu Arg Leu Arg Ser Lys Asp Arg  
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 Asp Leu Gly Ser Ser  
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&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

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Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu  
 20 25 30

Gly Asn Arg Arg Ala Leu Ile Leu Leu Gly Gln Met Gly Arg Ile Ser  
 35 40 45

Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Arg Ile Pro Gln Glu  
 50 55 60

Glu Phe Asp Gly Asn Gln Phe Gln Asp Ala Gln Ala Ile Ser Val Leu  
 65 70 75 80

His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser  
                                     85                                    90                                    95  
 Ser Ala Ala Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu  
                                     100                                    105                                    110  
 Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly  
                                     115                                    120                                    125  
 Val Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg  
                                     130                                    135                                    140  
 Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Ile Glu Arg Lys Tyr Ser  
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 Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser  
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 Phe Ser Thr Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp  
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&lt;211&gt; 189

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 8

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                                     20                                    25                                    30  
 Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg Ile Ser  
                                     35                                    40                                    45  
 Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu  
                                     50                                    55                                    60  
 Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala His Val Met Ser Val Leu  
   65                                    70                                    75                                    80  
 His Glu Met Leu Gln Gln Ile Phe Ser Leu Phe His Thr Glu Arg Ser  
                                     85                                    90                                    95  
 Ser Ala Ala Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu  
                                     100                                    105                                    110  
 Asn Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly  
                                     115                                    120                                    125  
 Val Glu Glu Thr Pro Leu Met Asn Val Asp Ser Ile Leu Ala Val Lys  
                                     130                                    135                                    140  
 Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser  
 145                                    150                                    155                                    160  
 Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser  
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Leu Ser Lys Ile Phe Gln Glu Arg Leu Arg Arg Lys Glu  
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Ser Pro Val Gly Ser Leu Gly Cys Asp Leu Pro Gln Asn His Gly Leu  
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Leu Ser Arg Asn Thr Leu Val Leu Leu His Gln Met Arg Arg Ile Ser  
 35 40 45

Pro Phe Leu Cys Leu Lys Asp Arg Arg Asp Phe Arg Phe Pro Gln Glu  
 50 55 60

Met Val Lys Gly Ser Gln Leu Gln Lys Ala His Val Met Ser Val Leu  
 65 70 75 80

His Glu Met Leu Gln Gln Ile Phe Ser Leu Phe His Thr Glu Arg Ser  
 85 90 95

Ser Ala Ala Trp Asn Met Thr Leu Leu Asp Gln Leu His Thr Glu Leu  
 100 105 110

His Gln Gln Leu Gln His Leu Glu Thr Cys Leu Leu Gln Val Val Gly  
 115 120 125

Glu Gly Glu Ser Ala Gly Ala Ile Ser Ser Pro Ala Leu Thr Leu Arg  
 130 135 140

Arg Tyr Phe Gln Gly Ile Arg Val Tyr Leu Lys Glu Lys Lys Tyr Ser  
 145 150 155 160

Asp Cys Ala Trp Glu Val Val Arg Met Glu Ile Met Lys Ser Leu Phe  
 165 170 175

Leu Ser Thr Asn Met Gln Glu Arg Leu Arg Ser Lys Asp Arg Asp Leu  
 180 185 190

Gly Ser Ser  
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Gly Asn Arg Arg Ala Trp Ile Leu Leu Ala Gln Met Gly Arg Ile Ser  
                   35                                  40                                  45  
 His Phe Ser Cys Leu Lys Asp Arg Tyr Asp Phe Gly Phe Pro Gln Glu  
           50                                  55                                  60  
 Val Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Ala Phe  
       65                                  70                                  75                                  80  
 His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asp Ser  
                   85                                  90                                  95  
 Ser Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Ile Glu Leu  
                   100                                  105                                  110  
 Phe Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Thr Gln Glu Val Gly  
           115                                  120                                  125  
 Val Glu Glu Ile Ala Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg  
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 Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Met Gly Lys Lys Tyr Ser  
       145                                  150                                  155                                  160  
 Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser  
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 Phe Ser Thr Asn Leu Gln Lys Gly Leu Arg Arg Lys Asp Met Pro Leu  
                   180                                  185                                  190  
 Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr Lys Ser Ile  
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 Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg  
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 Arg Ala Trp Ile Leu Leu Ala Gln Met Gly Arg Ile Ser His Phe Ser  
       225                                  230                                  235                                  240  
 Cys Leu Lys Asp Arg Tyr Asp Phe Gly Phe Pro Gln Glu Val Phe Asp  
                   245                                  250                                  255  
 Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Ala Phe His Glu Met  
                   260                                  265                                  270  
 Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala  
           275                                  280                                  285  
 Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Ile Glu Leu Phe Gln Gln  
       290                                  295                                  300  
 Leu Asn Asp Leu Glu Ala Cys Val Thr Gln Glu Val Gly Val Glu Glu  
       305                                  310                                  315                                  320  
 Ile Ala Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe  
                   325                                  330                                  335  
 Gln Arg Ile Thr Leu Tyr Leu Met Gly Lys Lys Tyr Ser Pro Cys Ala  
                   340                                  345                                  350



Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Phe Ser Thr  
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 20 25 30

Gly Ala Arg Glu Asn Leu Arg Leu Leu Ala Arg Met Asn Arg Leu Ser  
 35 40 45

Pro His Pro Cys Leu Gln Asp Arg Lys Asp Phe Gly Leu Pro Gln Glu  
 50 55 60

Met Val Glu Gly Asn Gln Leu Gln Lys Asp Gln Ala Ile Ser Val Leu  
 65 70 75 80

His Glu Met Leu Gln Gln Cys Phe Asn Leu Phe Tyr Thr Glu His Ser  
 85 90 95

Ser Ala Ala Trp Asn Thr Thr Leu Leu Glu Gln Leu Cys Thr Gly Leu  
 100 105 110

Gln Gln Gln Leu Glu Asp Leu Asp Ala Cys Leu Gly Pro Val Met Gly  
 115 120 125

Glu Lys Asp Ser Asp Met Gly Arg Met Gly Pro Ile Leu Thr Val Lys  
 130 135 140

Lys Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys Glu Tyr Ser  
 145 150 155 160

Asp Cys Ala Trp Glu Ile Ile Arg Met Glu Met Met Arg Ala Leu Ser  
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Ser Ser Thr Thr Leu Gln Lys Arg Leu Arg Lys Met Gly Gly Asp Leu  
 180 185 190

Asn Ser Leu  
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 Asp Ala Arg Glu Asn Leu Lys Leu Leu Glu Pro Met Asn Arg Leu Ser  
                     35                    40                    45  
 Pro His Ser Cys Leu Gln Asp Arg Lys Asp Phe Gly Leu Pro Gln Glu  
                     50                    55                    60  
 Met Val Glu Gly Asp Gln Leu Gln Lys Asp Gln Ala Phe Pro Val Leu  
                     65                    70                    75                    80  
 Tyr Glu Met Leu Gln Gln Thr Phe Asn Leu Phe His Thr Glu His Ser  
                     85                    90                    95  
 Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Cys Thr Gly Leu  
                     100                    105                    110  
 Gln Gln Gln Leu Glu Asp Leu Asp Thr Cys Cys Arg Gly Gln Val Met  
                     115                    120                    125  
 Gly Glu Glu Asp Ser Glu Leu Gly Asn Met Asp Pro Ile Val Thr Val  
                     130                    135                    140  
 Lys Lys Tyr Phe Gln Gly Ile Tyr Asp Tyr Leu Gln Glu Lys Gly Tyr  
                     145                    150                    155                    160  
 Ser Asp Cys Ala Trp Glu Ile Val Arg Val Glu Met Met Arg Ala Leu  
                     165                    170                    175  
 Thr Val Ser Thr Thr Leu Gln Lys Arg Leu Thr Lys Met Gly Gly Asp  
                     180                    185                    190  
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                     20                    25                    30  
 Lys Arg Glu Ile Phe Lys His Leu Gln Glu Ile Lys Lys Ile Pro Ser  
                     35                    40                    45  
 Gln Leu Cys Leu Lys Asp Arg Ile Asp Phe Lys Phe Pro Trp Lys Arg  
                     50                    55                    60  
 Glu Ser Ile Thr Gln Leu Gln Lys Asp Gln Ala Phe Pro Val Leu Tyr  
                     65                    70                    75                    80  
 Glu Met Leu Gln Gln Thr Phe Asn Leu Phe His Thr Glu His Ser Ser  
                     85                    90                    95

Ala Ala Trp Asn Thr Thr Leu Leu Asp Gln Leu Leu Ser Ser Leu Asp  
 100 105 110

Leu Gly Leu Arg Arg Leu Glu His Met Lys Lys Asp Asn Met Asp Cys  
 115 120 125

Pro His Val Gly Ser Ala Leu Arg Lys Tyr Phe Gln Gly Ile Gly Leu  
 130 135 140

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Ile Val Arg  
 145 150 155 160

Val Glu Ile Glu Arg Cys Phe Ser Leu Thr  
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&lt;211&gt; 212

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 14

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Gly Leu Leu Leu Val Leu Pro Ala Ala Phe Pro Ala Pro Val Pro Pro  
 20 25 30

Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His Arg Gln Pro Leu Thr  
 35 40 45

Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr Ile Leu Asp Gly Ile  
 50 55 60

Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser Asn Met Cys Glu Ser  
 65 70 75 80

Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn Leu Pro Lys Met Ala  
 85 90 95

Lys Glu Asp Gly Cys Phe Gln Ser Gly Phe Asn Glu Glu Thr Cys Leu  
 100 105 110

Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu Val Tyr Leu Glu Tyr  
 115 120 125

Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln Ala Arg Ala Val Gln  
 130 135 140

Met Ser Thr Lys Val Leu Ile Gln Phe Leu Gln Lys Lys Ala Lys Asn  
 145 150 155 160

Leu Asp Ala Ile Thr Thr Pro Asp Pro Thr Thr Asn Ala Ser Leu Leu  
 165 170 175

Thr Lys Leu Gln Ala Gln Asn Gln Trp Leu Gln Asp Met Thr Thr His  
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Leu Arg Gln Met  
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20 25 30  
Arg Ser Leu Ala Leu Cys Gln Lys Leu Leu Arg Gln Leu Pro Ser Thr  
35 40 45  
Pro Gln His Cys Leu Glu Ala Arg Met Asp Phe Gln Met Pro Glu Glu  
50 55 60  
Met Lys Gln Ala Gln Gln Phe Gln Lys Glu Asp Ala Ile Leu Val Ile  
65 70 75 80  
Tyr Glu Met Leu Gln Gln Ile Phe Asn Ile Leu Thr Arg Asp Phe Ser  
85 90 95  
Ser Thr Gly Trp Ser Glu Thr Ile Ile Glu Asp Leu Leu Glu Glu Leu  
100 105 110  
Tyr Glu Gln Met Asn His Leu Glu Pro Ile Gln Lys Glu Ile Met Gln  
115 120 125  
Lys Gln Asn Ser Thr Met Gly Asp Thr Thr Val Leu His Leu Arg Lys  
130 135 140  
Tyr Tyr Phe Asn Leu Val Gln Tyr Leu Lys Ser Lys Glu Tyr Asn Arg  
145 150 155 160  
Cys Ala Trp Thr Val Val Arg Val Gln Ile Leu Arg Asn Phe Ser Phe  
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29

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ggccgcggta ccttatttcc tcctgaatag agc

33

&lt;210&gt; 18

&lt;211&gt; 38

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 18

ggccgggata cgccatcatg agcaccaaac ctgatatg

38

&lt;210&gt; 19

&lt;211&gt; 33

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 19

ggccgcggta ccttatttcc tcctgaatag agc

33

&lt;210&gt; 20

&lt;211&gt; 156

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 20

Met	Thr	Tyr	Arg	Cys	Leu	Leu	Gln	Met	Val	Leu	Leu	Leu	Cys	Phe	Ser
1				5					10					15	

Thr	Thr	Ala	Leu	Ser	Arg	Ser	Tyr	Ser	Leu	Leu	Arg	Phe	Gln	Gln	Arg
			20					25					30		

Gln	Ser	Leu	Lys	Glu	Cys	Gln	Lys	Leu	Leu	Gly	Gln	Leu	Pro	Ser	Thr
		35					40					45			

Ser	Gln	His	Cys	Leu	Glu	Ala	Arg	Met	Asp	Phe	Gln	Met	Pro	Glu	Glu
	50					55					60				

Met	Lys	Gln	Glu	Gln	Gln	Phe	Gln	Lys	Glu	Asp	Ala	Ile	Leu	Val	Met
65					70					75					80

Tyr	Glu	Val	Leu	Gln	His	Ile	Phe	Gly	Ile	Leu	Thr	Arg	Asp	Phe	Ser
				85					90					95	

Ser	Thr	Gly	Trp	Asn	Ser	Thr	Thr	Glu	Asp	Thr	Ile	Val	Pro	His	Leu
			100					105					110		

Gly	Lys	Tyr	Tyr	Phe	Asn	Leu	Met	Gln	Tyr	Leu	Glu	Ser	Lys	Glu	Tyr
		115					120					125			

Asp	Arg	Cys	Ala	Trp	Thr	Val	Val	Gln	Val	Gln	Ile	Leu	Thr	Asn	Val
	130					135					140				

Ser	Phe	Leu	Met	Arg	Leu	Thr	Gly	Tyr	Val	Arg	Asp
145					150					155	

&lt;210&gt; 21

&lt;211&gt; 166

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 21

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Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
  1              5              10              15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
          20              25              30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
          35              40              45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
  50              55              60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
  65              70              75              80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
          85              90              95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
          100              105              110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
          115              120              125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
          130              135              140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
          145              150              155              160

Thr Gly Tyr Leu Gly Asn
          165

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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/16424

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 15/12, 15/20; C07K 14/435, 14/555; A61K 38/17, 38/21  
US CL : Please See Extra Sheet.  
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5, 23.52; 435/320.1, 325, 252.3, 69.1, 69.51; 530/351, 387.1; 514/2; 424/85.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	US 5,710,027 A (HAUPTMANN et al.) 20 January 1998, particularly residues 131-142 of the IFN-omegal sequence depicted in SEQ ID NO: 9 and the corresponding portion of the cDNA sequence in SEQ ID NO: 8.	1, 2, 6, 7, 18 ----- 9-13, 16 ----- 3, 4, 8, 14, 15, 17, 19, 20
X, P	Database dbEST, Accession No. AI155872, MARRA, M. et al. 'The WashU-HHMI Mouse EST Project', database record, 30 September 1998, especially the subsequences AGTGGAAATAAGAAGATGTTT and AGAATCACCCACCTTCAAGCAAGAA.	1, 2, 6, 9-12

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

•	Special categories of cited documents:	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A*	document defining the general state of the art which is not considered to be of particular relevance		
*E*	earlier document published on or after the international filing date	*X*	document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*L*	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y*	document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*O*	document referring to an oral disclosure, use, exhibition or other means		
*P*	document published prior to the international filing date but later than the priority date claimed	*G*	document member of the same patent family

Date of the actual completion of the international search

27 AUGUST 1999

Date of mailing of the international search report

05 NOV 1999

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

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Authorized officer

DAVID L. FITZGERALD

Telephone No. (703) 308-0196

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/16424

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 5  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
  
Please See Extra Sheet.
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.



# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/16424

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

536/23.5, 23.52; 435/320.1, 325, 252.3, 69.1, 69.51; 530/351, 387.1; 514/2; 424/85.4

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

KEYWORD DATABASE: Medline (search terms: keratinocyte, interferon/IFN)

SEQUENCE DATABASES: GenBank/EMBL/DBJ, dbEST, GeneSeq, SwissProt, PIR, USPTO-Issued

## BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE

2. Where no meaningful search could be carried out, specifically:

Claim 5 cannot be construed as to alternatives (d), (e), and (f) because they recite contradictory and mutually exclusive limitations. In particular, the "polypeptide encoded by the human cDNA in clone HKAPI15" is necessarily a full-length polypeptide and hence cannot be "lacking 1 [to] 58 amino acids from its N-terminus." Alternatively, to the extent that the residues which the reference polypeptide is "lacking" are not those encoded by the clone, it is not clear what intact reference polypeptide marks the metes and bounds of the claim. Claim 5 is accordingly so indefinite that this Authority can draw no meaningful comparison between the subject matter it embraces and the prior art.



## Keratinocyte Derived Interferon

1 CCA CGC GTC CGG GAT TTT TTA GCT TGC AAA AAA AAT GAG CAC CAA ACC TTA TAT GAT TCA 60  
 1 M S T K P D M L Q 9  
 61 AAA GTC TTT GTC GCT TTA GAT GCT TAT GAG TAT ATT CAT TGC TGG CAC GCT ATC GCT GGA 120  
 9 K C L N L E I L M G I F I A Q T L E L D 29  
 121 CTG TAA CTT ACT GAA CGT TCA GCT GAG AAG AGT CAC CTG CCA AAA TCT GAG ACA TCT GAG 180  
 29 C N L L N V H L R R V T W Q N L R H L S 49  
 181 TAG TAT GAG CAA TTC ATT TCC TGT AGA ATG TCT ACG AGA AAA CAT ACC TTT TGA GTT GCG 240  
 49 S M S M S P P V E C L K E N I A P E L P 69  
 241 CCA AGA GTT TCT GCA ATA CAC CCA ACC TAT CAA GAG GGA CAT CAA GAA GGC CTT CTA TGA 300  
 69 Q E F L Q Y T Q P H K R D I R K A P Y E 89  
 301 AAT GTC CCT ACA GGC CTT CAA CAT CTT CAG CCA ACA CAC CTT CAA ATA TTG GAA AGA GAG 360  
 89 M S L Q A F N I F S Q H T P E Y W K E R 109  
 361 ACA CTT CAA ACA AAT CCA AAT AGC ACT TCA TCA GCA AGC AGA GTA CTT GAA CCA ATG CTT 420  
 109 H L K Q I Q I G L D Q Q A E Y L N Q C L 129  
 421 GGA GGA AGA CCA GAA TGA AAA TGA AGA CAT GAA AGA AAT GAA AGA GAA TGA GAT GAA ACC 480  
 129 E E D E N E N E D M K E M K E N E M K P 149  
 481 CTC AGA AGC CAG GGT GGC CCA GCT GAG CAG CCT GGA ACT GAG GAG ATA TTT CCA CAG GAT 540  
 149 S E A R V P Q L S S L E L R R Y F H R I 169  
 541 AGA CAA TTT CTT GAA AGA AAA GAA ATA CAG TGA CTG TGC CTG GGA GAT TGT CCG AGT GGA 600  
 169 D N F L K E K K Y S D E A W E I V R V E 189  
 601 AAT CAG AAG ATG TTT GTA TTA CTT TTA CAA AAT TAC AGC TCT ATT CAG GAG GAA ATA AGA 660  
 189 I R R C L Y Y F Y K Y T A L F R R K ' 207  
 661 ATC ATC TAC CTT CAA CCA AGA AAT AAC AGA GAT TGT GGC TAC GCA AAT GCA CCA AAA AAG 720  
 721 GGT GAA ATA TAT CTG AAA TGT ACC TGG TTC TGC CCT TGG AAG CCA CTT CTT GCT CAT GCC 780  
 781 ACT AAC AGC ATG CTG CCA AAC TGT TCA GAT TCA AGA TTA TTC CAA GCG CAG GGC CCA AAT 840  
 841 GTT ATA GGC AAA GAA AGT CTT ATG ATA AAA GTG AGG CAA AAT TCA GCC AAG AAG TTA GAA 900  
 901 GAG ATG TTT AAA ACA ACA AGA ACA AAT TGT GGA TCA TGS TAT ATG CAG GCT ATC AGC AGA 960  
 961 AGG ATC AGA CAA TAA AAT GAG TTA GTG CAA ACC ATT TAA TAA AAA TAA CTA TCA GCA GAG 1020  
 1021 TGG TTC CAG ATT AAA AAT AGT ACT ACA AGC TTG TAA AGG AGT TAC GAC ATG CAA GCT ACT 1080  
 1081 GAG CAT AAA ATA TAT ACT TGC TAT TTT TCA TGA CTT TCT CTA ATA AAG TCT TTG ACT GTT 1140  
 1141 CTC TCT AAT AAA AAA AAA AAA AAA AAA AAA 1170

FIG. 1

HKAPI15	10	XCLWLEILXGIFIA...TSLDCNLLNVH...LRVITWQNLRHLSMSMS	54
INF-omega	1	MALLFPLLAALVMTESYSPVGSIGCDLPQNHGLLSRNTLVLLHQMRRIS..	48
HKAPI15	55	FPVRCLRENIAPELPQEFLOYTOPMKRDIKKAFYEMSLOQAFNIF.SOHTF	103
INF-omega	49	.PFLCLKDRDRDFRFPQEMVYKGSQLOKAYVMSVLHEMLQQIFSLFHTERSG	97
HKAPI15	104	KYWKERHLKQIQIGLDQQAAYLNQCLESDEENEDMKEMKENEMKPSGAR	152
INF-omega	98	AAWNMTLLDQLHTELHQQLOHLETCLLQVVGEGESAGAISS.....	138
HKAPI15	154	VPQISSLELRYPHRIDNPLKEKKYSDCAWEIVRVEIRRCLYYFYKFTAL	202
INF-omega	139	....PALTLRRYFQGIKRVYLKEKKYSDCAWEVVRMEIMKSLFLSTNWQER	184
HKAPI15	204	FRRK 207	
INF-omega	185	LRSK 188	

**FIG. 2**

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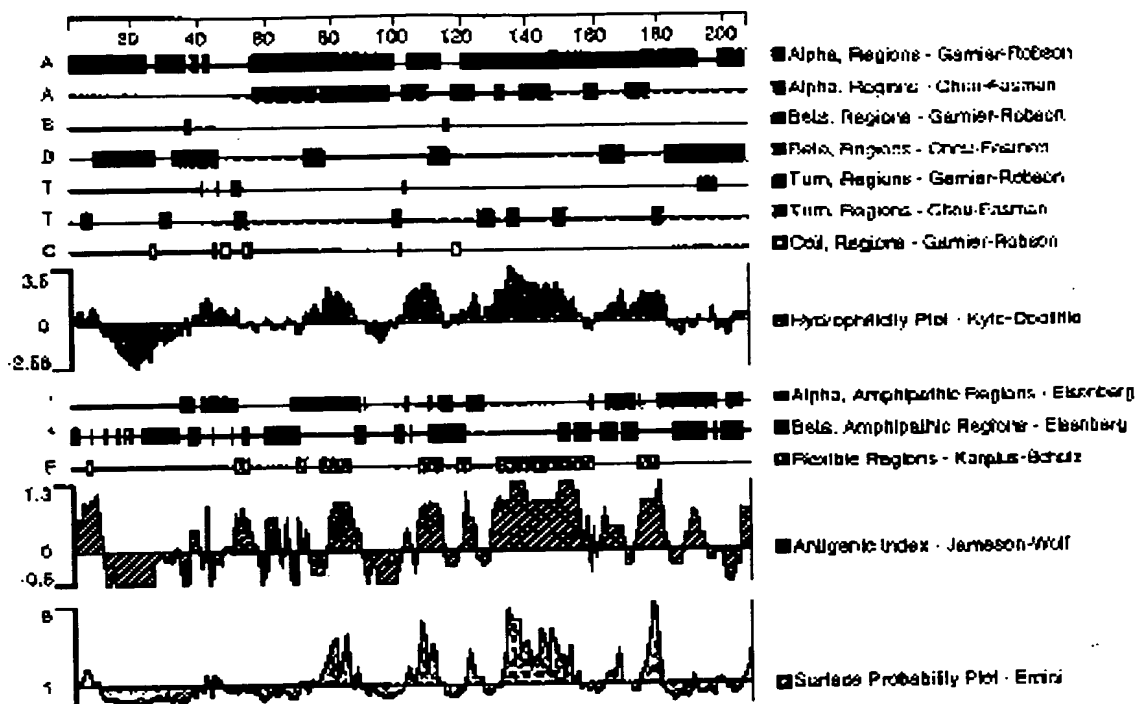


FIG. 3

417

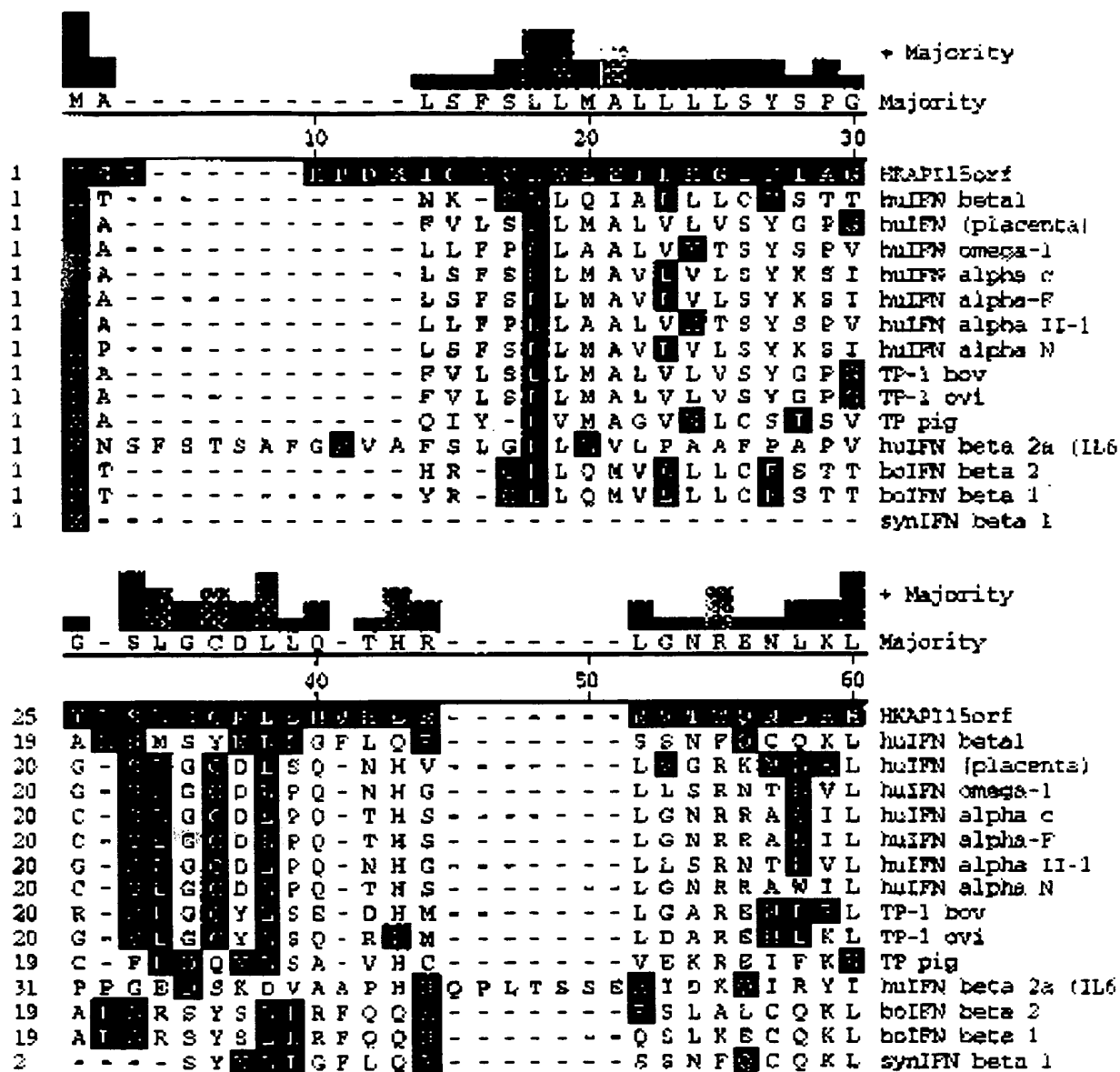


FIG. 4A

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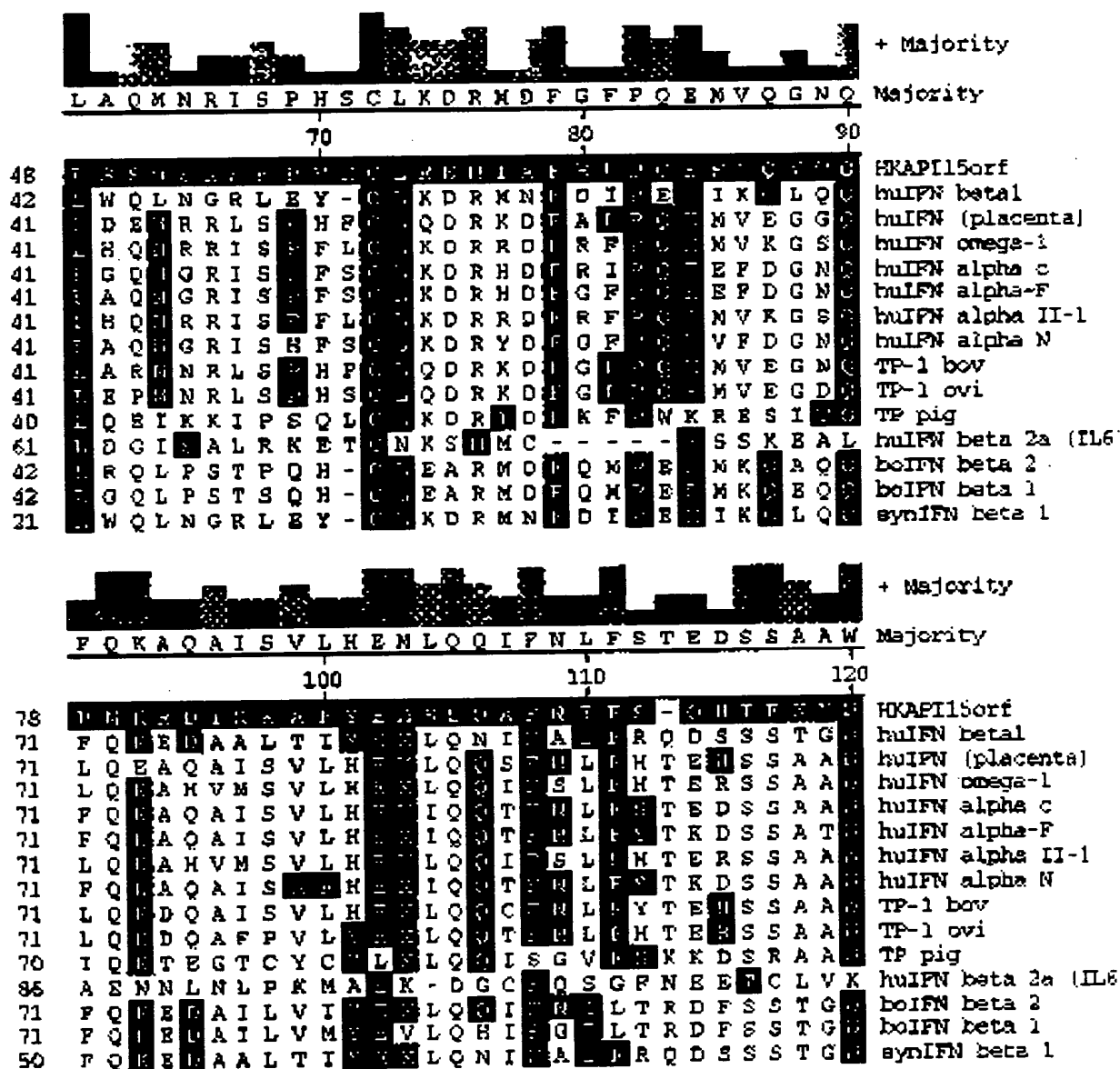


FIG. 4B

NETTLEOLLTELYOOLNOLBACLEQVMGEE Majority

S - - - - - A L G N V D S I L A L R K Y F Majority

	160	170	180	
137	E T K E F M E F H E R E P S D A R F L Q G A I E E E E	KKAPI15orf		
131	D F - - - - - - - - - T V G K L M S S L H - K E Y	huIFN beta1		
131	D S - - - - - - - - - L G R T G P T I A - K E Y	huIFN (placenta)		
131	S - - - - - - - - - S G A I S S P A T T B E Y	huIFN omega-1		
131	T - - - - - - - - - P L M N E D I L A V P K -	huIFN alpha c		
131	S - - - - - - - - - P L M N V D I I A V K X	huIFN alpha-F		
131	I S - - - - - - - - - G A I S S P A T L R E Y	huIFN alpha II-1		
131	I I - - - - - - - - - L M N E D I I A V R K -	huIFN alpha N		
131	D S - - - - - - - - - D M G R M G P I L T V K K	TP-1 bov		
131	D S - - - - - - - - - E L G N W D P I V T V K K	TP-1 ovi		
125	N M - - - - - - - - - D C P H V G A L - - K Y	TP pig		
145	M S T E V L I Q F L Q E K A K N L D A I T T P D P T N -	huIFN beta 2a (IL6)		
131	M S - - - - - - - - - T M G D - T T V I H E K Y	boIFN beta 2		
131	M S - - - - - - - - - T T E D - T I V P H G K Y	boIFN beta 1		
110	D F - - - - - - - - - T V G K L M S S L H - K E Y	synIFN beta 1		

**FIG. 4C**



717

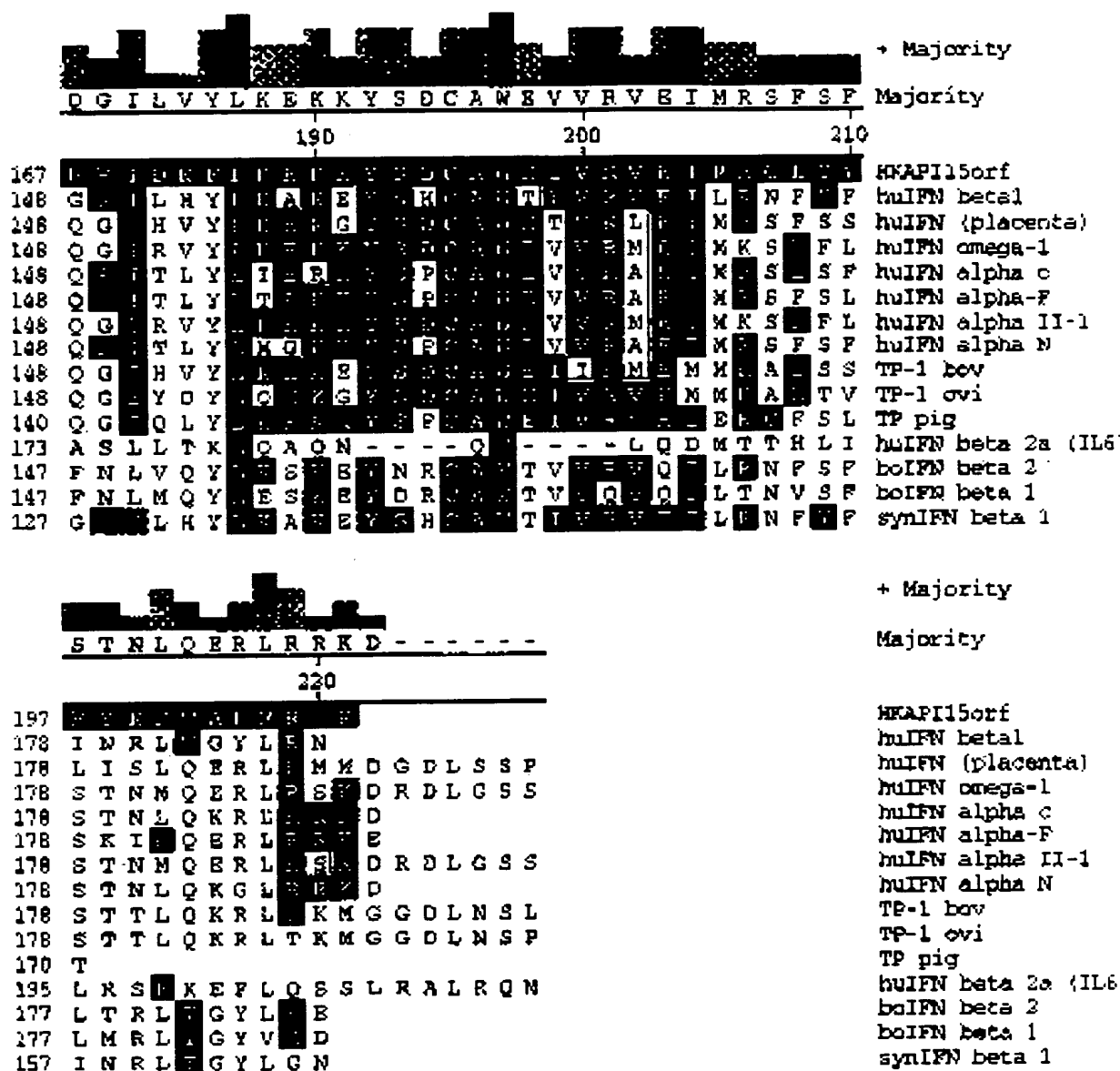


FIG. 4D

## SEQUENCE LISTING

&lt;110&gt; Human Genome Sciences, Inc., et al.

&lt;120&gt; Keratinocyte Derived Interferon

&lt;130&gt; PF482

&lt;140&gt; Unassigned

&lt;141&gt; 1999-07-21

&lt;150&gt; 60/093,643

&lt;151&gt; 1998-07-21

&lt;160&gt; 21

&lt;170&gt; PatentIn Ver. 2.0

&lt;210&gt; 1

&lt;211&gt; 1170

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (35)...(655)

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                                   1           5

att caa aag tgt ttg tgg ctt gag atc ctt atg ggt ata ttc att gct 103
ile gln lys cys leu trp leu glu ile leu met gly ile phe ile ala
      10           15           20

ggc acc cta tcc ctg gac tgt aac tta ctg aac gtt cac ctg aga aga 151
gly thr leu ser leu asp cys asn leu leu asn val his leu arg arg
      25           30           35

gtc acc tgg caa aat ctg aga cat ctg agt agt atg agc aat tca ttt 199
val thr trp gln asn leu arg his leu ser ser met ser asn ser phe
      40           45           50           55

cct gta gaa tgt cta cga gaa aac ata gct ttt gag ttg ccc caa gag 247
pro val glu cys leu arg glu asn ile ala phe glu leu pro gln glu
      60           65           70

ttt ctg caa tac acc caa cct atg aag agg gac atc aag aag gcc ttc 295
phe leu gln tyr thr gln pro met lys arg asp ile lys lys ala phe
      75           80           85

tat gaa atg tcc cta cag gcc ttc aac atc ttc agc caa caa acc ttc 343
tyr glu met ser leu gln ala phe asn ile phe ser gln his thr phe
      90           95          100

aaa tat tgg aaa gag aga cac ctg aaa caa atc caa ata gga ctt gat 391
lys tyr trp lys glu arg his leu lys gln ile gln ile gly leu asp
     105           110           115

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Gln Gln Ala Glu Tyr Leu Asn Gln Cys Leu Glu Glu Asp Glu Asn Glu  
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 Asn Glu Asp Met Lys Glu Met Lys Glu Asn Glu Met Lys Pro Ser Glu  
 140 145 150

gcc agg gtc ccc cag ctg agc agc ctg gaa ctg agg aga tat ttc cac 535  
 Ala Arg Val Pro Gln Leu Ser Ser Leu Glu Leu Arg Arg Tyr Phe His  
 155 160 165

agg ata gac aat ttc atg aaa gaa aag aaa tac agt gac tgt gcc tgg 583  
 Arg Ile Asp Asn Phe Leu Lys Glu Lys Lys Tyr Ser Asp Cys Ala Trp  
 170 175 180

gag att gtc cga ggg gaa atc aga aga tgt ttg tat tac ttt tac aaa 631  
 Glu Ile Val Arg Val Glu Ile Arg Arg Cys Leu Tyr Tyr Phe Tyr Lys  
 185 190 195

ttt acc gcl ctg ttc agg agg aaa taagaatcat ctaccttcaa gcaagaatta 685  
 Phe Thr Ala Leu Phe Arg Arg Lys  
 200 205

acagagattg tggctacgca atgcaccaa aaaggggtga aatatactcg aatgtacct 745  
 gggtctgcc ttggaagcca ctctctgtc atgccactaa cagcatgctg ccaaatgtgt 805  
 cagattcaag attattccaa ggcaggggoc caaatgttat agccaaagaa agtcttatga 865  
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 attgtggatc atggtatatg caggctatca gcagaaggat cagacataa aatgagttag 985  
 tgcacacac ttgtaaaaa taactatcag cagagttgtt ccagattcaa aatagtacta 1045  
 caagcttgta aaggagtttag gacatgcaag ctactgagca taaatataat acttgctatt 1105  
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 aaaaa 1170

&lt;210&gt; 2

&lt;211&gt; 207

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 2

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Leu Met Gly Ile Phe Ile Ala Gly Thr Leu Ser Leu Asp Cys Asn Leu  
 20 25 30

Leu Asn Val His Leu Arg Arg Val Thr Trp Gln Asn Leu Arg His Leu  
 35 40 45

Ser Ser Met Ser Asn Ser Phe Pro Val Glu Cys Leu Arg Glu Asn Ile  
 50 55 60

Ala Phe Glu Leu Pro Gln Glu Phe Leu Gln Tyr Thr Gln Pro Met Lys

65	70	75	80
Arg Asp Ile Lys Lys Ala Phe Tyr Glu Met Ser Leu Gln Ala Phe Asn	85	90	95
Ile Phe Ser Gln His Thr Phe Lys Tyr Trp Lys Glu Arg His Leu Lys	100	105	110
Gln Ile Gln Ile Gly Leu Asp Gln Gln Ala Glu Tyr Leu Asn Gln Cys	115	120	125
Leu Glu Glu Asp Glu Asn Glu Asn Glu Asp Met Lys Glu Met Lys Glu	130	135	140
Asn Glu Met Lys Pro Ser Glu Ala Arg Val Pro Gln Leu Ser Ser Leu	145	150	155
Glu Leu Arg Arg Tyr Phe His Arg Ile Asp Asn Phe Leu Lys Glu Lys	165	170	175
Lys Tyr Ser Asp Cys Ala Trp Glu Ile Val Arg Val Glu Ile Arg Arg	180	185	190
Cys Leu Tyr Tyr Phe Tyr Lys Phe Thr Ala Leu Phe Arg Arg Lys	195	200	205
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<211> 238			
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<213> Homo sapiens			
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Leu Ser Arg Asn Thr Leu Val Leu Leu His Gln Met Arg Arg Ile Ser	35	40	45
Pro Phe Leu Cys Leu Lys Asp Arg Arg Asp Phe Arg Phe Pro Gln Glu	50	55	60
Met Val Lys Gly Ser Gln Leu Gln Lys Ala His Val Met Ser Val Leu	65	70	75
His Glu Met Leu Gln Gln Ile Phe Ser Leu Phe His Thr Glu Arg Ser	85	90	95
Ser Ala Ala Trp Asn Met Thr Leu Leu Asp Glu Leu His Thr Glu Leu	100	105	110
His Gln Gln Leu Gln His Leu Glu Thr Cys Leu Leu Gln Val Val Gly	115	120	125
Glu Gly Glu Ser Ala Gly Ala Ile Ser Ser Val Pro Gln Leu Ser Ser	130	135	140
Leu Glu Leu Arg Arg Tyr Phe His Arg Ile Asp Asn Phe Leu Lys Glu			

145                      150                      155                      160  
 Lys Lys Tyr Ser Asp Cys Ala Trp Glu Ile Val Arg Val Glu Ile Arg  
                                  165                      170                      175  
 Arg Cys Leu Tyr Tyr Phe Tyr Lys Phe Thr Ala Leu Pro Ala Leu Thr  
                                  180                      185                      190  
 Leu Arg Arg Tyr Phe Gln Gly Ile Arg Val Tyr Leu Lys Glu Lys Lys  
                                  195                      200                      205  
 Tyr Ser Asp Cys Ala Trp Glu Val Val Arg Met Glu Ile Met Lys Ser  
                                  210                      215                      220  
 Leu Phe Leu Ser Thr Asn Met Gln Glu Arg Leu Arg Ser Lys  
                                  225                      230                      235

&lt;310&gt; 4

&lt;211&gt; 187

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 4

Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser  
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 Thr Thr Ala Leu Ser Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg  
                                   20                                  25                                  30  
 Ser Ser Asn Phe Gln Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg  
                                   35                                  40                                  45  
 Leu Glu Tyr Cys Leu Lys Asp Arg Met Asn Phe Asp Ile Pro Gln Glu  
                                   50                                  55                                  60  
 Ile Lys Gln Leu Gln Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile  
   65                                  70                                  75                                  80  
 Tyr Glu Met Leu Gln Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser  
                                   85                                  90                                  95  
 Ser Thr Gly Trp Asn Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val  
                                  100                                 105                                 110  
 Tyr His Gln Ile Asn His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu  
                                  115                                 120                                 125  
 Lys Glu Asp Phe Thr Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys  
                                  130                                 135                                 140  
 Arg Tyr Tyr Gly Arg Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser  
  145                                 150                                 155                                 160  
 His Cys Ala Trp Thr Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr  
                                  165                                 170                                 175  
 Phe Ile Asn Arg Leu Thr Gly Tyr Leu Arg Asn  
                                  180                                 185

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 <213> Homo sapiens

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 Val Gly Arg Lys Asn Leu Arg Leu Leu Asp Glu Met Arg Arg Leu Ser  
 35 40 45  
 Pro His Phe Cys Leu Gln Asp Arg Lys Asp Phe Ala Leu Pro Gln Glu  
 50 55 60  
 Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile Ser Val Leu  
 65 70 75 80  
 His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr Glu His Ser  
 85 90 95  
 Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Pro Cys Arg Thr Gly Leu  
 100 105 110  
 His Gln Gln Leu Asp Asn Leu Asp Ala Cys Leu Gly Gln Val Met Gly  
 115 120 125  
 Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Leu Ala Leu Lys Arg  
 130 135 140  
 Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys Gly Tyr Ser Asp  
 145 150 155 160  
 Cys Ala Trp Glu Thr Val Arg Leu Glu Ile Met Arg Ser Phe Ser Ser  
 165 170 175  
 Leu Ile Ser Leu Gln Glu Arg Leu Arg Met Met Asp Gly Asp Leu Ser  
 180 185 190  
 Ser Pro

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 <213> Homo sapiens

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 Met Ala Leu Leu Phe Pro Leu Leu Ala Ala Leu Val Met Thr Ser Tyr  
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 Ser Pro Val Gly Ser Leu Gly Cys Asp Leu Pro Gln Asn His Gly Leu  
 20 25 30  
 Leu Ser Arg Asn Thr Leu Val Leu Leu His Gln Met Arg Arg Ile Ser  
 35 40 45

Pro Phe Leu Cys Leu Lys Asp Arg Arg Asp Phe Arg Phe Pro Gln Glu  
 50 55 60  
 Met Val Lys Gly Ser Gln Leu Gln Lys Ala His Val Met Ser Val Leu  
 65 70 75 80  
 His Glu Met Leu Gln Gln Ile Phe Ser Leu Phe His Thr Glu Arg Ser  
 85 90 95  
 Ser Ala Ala Trp Asn Met Thr Leu Leu Asp Gln Leu His Thr Glu Leu  
 100 105 110  
 His Gln Gln Leu Gln His Leu Gln Thr Cys Leu Leu Gln Val Val Gly  
 115 120 125  
 Glu Gly Glu Ser Ala Gly Ala Ile Ser Ser Val Pro Gln Leu Ser Ser  
 130 135 140  
 Leu Glu Leu Arg Arg Tyr Phe His Arg Ile Asp Asn Phe Leu Lys Glu  
 145 150 155 160  
 Lys Lys Tyr Ser Asp Cys Ala Trp Glu Ile Val Arg Val Glu Ile Arg  
 165 170 175  
 Arg Cys Leu Tyr Tyr Phe Tyr Lys Phe Thr Ala Leu Pro Ala Leu Thr  
 180 185 190  
 Leu Arg Arg Tyr Phe Gln Gly Ile Arg Val Tyr Leu Lys Glu Lys Lys  
 195 200 205  
 Tyr Ser Asp Cys Ala Trp Glu Val Val Arg Met Glu Ile Met Lys Ser  
 210 215 220  
 Leu Phe Leu Ser Thr Asn Met Gln Glu Arg Leu Arg Ser Lys Asp Arg  
 225 230 235 240  
 Asp Leu Gly Ser Ser  
 245

<210> 7  
 <211> 189  
 <212> PRT  
 <213> Homo sapiens

<400> 7  
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 Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu  
 20 25 30  
 Gly Asn Arg Arg Ala Leu Ile Leu Leu Gly Gln Met Gly Arg Ile Ser  
 35 40 45  
 Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Arg Ile Pro Gln Glu  
 50 55 60  
 Glu Phe Asp Gly Asn Gln Phe Gln Asp Ala Gln Ala Ile S r Val Leu  
 65 70 75 80

His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser  
                                     85                                    90                                    95  
 Ser Ala Ala Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu  
                                     100                                    105                                    110  
 Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly  
                                     115                                    120                                    125  
 Val Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg  
                                     130                                    135                                    140  
 Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Ile Glu Arg Lys Tyr Ser  
                                     145                                    150                                    155                                    160  
 Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser  
                                     165                                    170                                    175  
 Phe Ser Thr Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp  
                                     180                                    185

&lt;210&gt; H

&lt;211&gt; 189

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; H

Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr  
                                     1                                    5                                    10                                    15  
 Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu  
                                     20                                    25                                    30  
 Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg Ile Ser  
                                     35                                    40                                    45  
 Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu  
                                     50                                    55                                    60  
 Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala His Val Met Ser Val Leu  
                                     65                                    70                                    75                                    80  
 His Glu Met Leu Gln Gln Ile Phe Ser Leu Phe His Thr Glu Arg Ser  
                                     85                                    90                                    95  
 Ser Ala Ala Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu  
                                     100                                    105                                    110  
 Asn Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly  
                                     115                                    120                                    125  
 Val Glu Glu Thr Pro Leu Met Asn Val Asp Ser Ile Leu Ala Val Lys  
                                     130                                    135                                    140  
 Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser  
                                     145                                    150                                    155                                    160  
 Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser  
                                     165                                    170                                    175



Leu Ser Lys Ile Phe Gln Glu Arg Leu Arg Arg Lys Glu  
180 185

<210> 9  
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<213> Homo sapiens

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Ser Pro Val Gly Ser Leu Gly Cys Asp Leu Pro Glu Asn His Gly Leu  
20 25 30

Leu Ser Arg Asn Thr Leu Val Leu Leu His Gln Met Arg Arg Ile Ser  
35 40 45

Pro Phe Leu Cys Leu Lys Asp Arg Arg Asp Phe Arg Phe Pro Gln Glu  
50 55 60

Met Val Lys Gly Ser Gln Leu Gln Lys Ala His Val Met Ser Val Leu  
65 70 75 80

His Glu Met Leu Gln Gln Ile Phe Ser Leu Phe His Thr Glu Arg Ser  
85 90 95

Ser Ala Ala Trp Asn Met Thr Leu Leu Asp Gln Leu His Thr Glu Leu  
100 105 110

His Gln Gln Leu Gln His Leu Glu Thr Cys Leu Leu Gln Val Val Gly  
115 120 125

Glu Gly Glu Ser Ala Gly Ala Ile Ser Ser Pro Ala Leu Thr Leu Arg  
130 135 140

Arg Tyr Phe Gln Gly Ile Arg Val Tyr Leu Lys Glu Lys Lys Tyr Ser  
145 150 155 160

Asp Cys Ala Trp Glu Val Val Arg Met Glu Ile Met Lys Ser Leu Phe  
165 170 175

Leu Ser Thr Asn Met Gln Glu Arg Leu Arg Ser Lys Asp Arg Asp Leu  
180 185 190

Gly Ser Ser  
195

<210> 10  
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<213> Homo sapiens

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Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu  
20 25 30

Gly Asn Arg Arg Ala Trp Ile Leu Leu Ala Gln Met Gly Arg Ile Ser  
 35 40 45  
 His Phe Ser Cys Leu Lys Asp Arg Tyr Asp Phe Gly Phe Pro Gln Glu  
 50 55 60  
 Val Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Ala Phe  
 65 70 75 80  
 His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asp Ser  
 85 90 95  
 Ser Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Ile Glu Leu  
 100 105 110  
 Phe Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Thr Gln Glu Val Gly  
 115 120 125  
 Val Glu Glu Ile Ala Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg  
 130 135 140  
 Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Met Gly Lys Lys Tyr Ser  
 145 150 155 160  
 Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser  
 165 170 175  
 Phe Ser Thr Asn Leu Gln Lys Gly Leu Arg Arg Lys Asp Met Pro Leu  
 180 185 190  
 Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr Lys Ser Ile  
 195 200 205  
 Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg  
 210 215 220  
 Arg Ala Trp Ile Leu Leu Ala Gln Met Gly Arg Ile Ser His Phe Ser  
 225 230 235 240  
 Cys Leu Lys Asp Arg Tyr Asp Phe Gly Phe Pro Gln Glu Val Phe Asp  
 245 250 255  
 Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Ala Phe His Glu Met  
 260 265 270  
 Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala  
 275 280 285  
 Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Ile Glu Leu Phe Gln Gln  
 290 295 300  
 Leu Asn Asp Leu Glu Ala Cys Val Thr Gln Glu Val Gly Val Glu Glu  
 305 310 315 320  
 Ile Ala Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe  
 325 330 335  
 Gln Arg Ile Thr Leu Tyr Leu Met Gly Lys Lys Tyr Ser Pro Cys Ala  
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Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Phe Ser Thr  
 355 360 365

Asn Leu Gln Lys Gly Leu Arg Arg Lys Asp  
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<210> 11

<211> 195

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<213> Homo sapiens

<400> 11

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 1 5 10 15

Gly Pro Gly Arg Ser Leu Gly Cys Tyr Leu Ser Glu Asp His Met Leu  
 20 25 30

Gly Ala Arg Glu Asn Leu Arg Leu Leu Ala Arg Met Asn Arg Leu Ser  
 35 40 45

Pro His Pro Cys Leu Gln Asp Arg Lys Asp Phe Gly Leu Pro Gln Glu  
 50 55 60

Met Val Glu Gly Asn Gln Leu Gln Lys Asp Gln Ala Ile Ser Val Leu  
 65 70 75 80

His Glu Met Leu Gln Gln Cys Phe Asn Leu Phe Tyr Thr Glu His Ser  
 85 90 95

Ser Ala Ala Trp Asn Thr Thr Leu Leu Glu Gln Leu Cys Thr Gly Leu  
 100 105 110

Gln Gln Gln Leu Glu Asp Leu Asp Ala Cys Leu Gly Pro Val Met Gly  
 115 120 125

Glu Lys Asp Ser Asp Met Gly Arg Met Gly Pro Ile Leu Thr Val Lys  
 130 135 140

Lys Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys Glu Tyr Ser  
 145 150 155 160

Asp Cys Ala Trp Glu Ile Ile Arg Met Glu Met Met Arg Ala Leu Ser  
 165 170 175

Ser Ser Thr Thr Leu Gln Lys Arg Leu Arg Lys Met Gly Gly Asp Leu  
 180 185 190

Asn Ser Leu  
 195

<210> 12

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<212> PRT

<213> Homo sapiens

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Met Ala Phe Val Leu Ser Leu Leu Met Ala Leu Val Leu Val Ser Tyr  
 1 5 10 15

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<211> 170
<212> PRT
<213> Homo sapiens
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Ser Val Cys Phe Leu Asp Gln Asn Leu Ser Ala Val His Cys Val Glu
      20              25              30
Lys Arg Gln Ile Phe Lys His Leu Gln Glu Ile Lys Lys Ile Pro Ser
      35              40              45
Gln Leu Cys Leu Lys Asp Arg Ile Asp Phe Lys Phe Pro Trp Lys Arg
      50              55              60
Glu Ser Ile Thr Gln Leu Gln Lys Asp Gln Ala Phe Pro Val Leu Tyr
      65              70              75              80
Glu Met Leu Gln Gln Thr Phe Asn Leu Phe His Thr Glu His Ser Ser
      85              90              95

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Ala Ala Trp Asn Thr Thr Leu Leu Asp Gln Leu Leu Ser Ser Leu Asp  
100 105 110

Leu Gly Leu Arg Arg Leu Glu His Met Lys Lys Asp Asn Met Asp Cys  
115 120 125

Pro His Val Gly Ser Ala Leu Arg Lys Tyr Phe Gln Gly Ile Gly Leu  
130 135 140

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Ile Val Arg  
145 150 155 160

Val Glu Ile Glu Arg Cys Phe Ser Leu Thr  
165 170

<210> 14

<211> 212

<212> PRT

<213> Homo sapiens

<400> 24

Met Asn Ser Phe Ser Thr Ser Ala Phe Gly Pro Val Ala Phe Ser Leu  
1 5 10 15

Gly Leu Leu Leu Val Leu Pro Ala Ala Phe Pro Ala Pro Val Pro Pro  
20 25 30

Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His Arg Gln Pro Leu Thr  
35 40 45

Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr Ile Leu Asp Gly Ile  
50 55 60

Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser Asn Met Cys Glu Ser  
65 70 75 80

Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn Leu Pro Lys Met Ala  
85 90 95

Lys Glu Asp Gly Cys Phe Gln Ser Gly Phe Asn Glu Glu Thr Cys Leu  
100 105 110

Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu Val Tyr Leu Glu Tyr  
115 120 125

Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln Ala Arg Ala Val Gln  
130 135 140

Met Ser Thr Lys Val Leu Ile Gln Phe Leu Gln Lys Lys Ala Lys Asn  
145 150 155 160

Leu Asp Ala Ile Thr Thr Pro Asp Pro Thr Thr Asn Ala Ser Leu Leu  
165 170 175

Thr Lys Leu Gln Ala Gln Asn Gln Trp Leu Gln Asp Met Thr Thr His  
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Leu Ile Leu Arg S r Phe Lys Glu Phe Leu Gln Ser Ser Leu Arg Ala  
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Leu Arg Gln Met  
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20 25 30  
Arg Ser Leu Ala Leu Cys Gln Lys Leu Leu Arg Gln Leu Pro Ser Thr  
35 40 45  
Pro Gln His Cys Leu Glu Ala Arg Met Asp Phe Gln Met Pro Glu Glu  
50 55 60  
Met Lys Gln Ala Gln Gln Phe Gln Lys Glu Asp Ala Ile Leu Val Ile  
65 70 75 80  
Tyr Glu Met Leu Gln Gln Ile Phe Asn Ile Leu Thr Arg Asp Phe Ser  
85 90 95  
Ser Thr Gly Trp Ser Glu Thr Ile Ile Glu Asp Leu Leu Glu Glu Leu  
100 105 110  
Tyr Glu Gln Met Asn His Leu Glu Pro Ile Gln Lys Glu Ile Met Gln  
115 120 125  
Lys Gln Asn Ser Thr Met Gly Asp Thr Thr Val Leu His Leu Arg Lys  
130 135 140  
Tyr Tyr Phe Asn Leu Val Gln Tyr Leu Lys Ser Lys Glu Tyr Asn Arg  
145 150 155 160  
Cys Ala Trp Thr Val Val Arg Val Gln Ile Leu Arg Asn Phe Ser Phe  
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29

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33

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38

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Thr Thr Ala Leu Ser Arg Ser Tyr Ser Leu Leu Arg Phe Gln Gln Arg  
20 25 30  
Gln Ser Leu Lys Gly Cys Gln Lys Leu Leu Gly Gln Leu Pro Ser Thr  
35 40 45  
Ser Gln His Cys Leu Glu Ala Arg Met Asp Phe Gln Met Pro Glu Glu  
50 55 60  
Met Lys Gln Glu Gln Gln Phe Gln Lys Glu Asp Ala Ile Leu Val Met  
65 70 75 80  
Tyr Glu Val Leu Gln His Ile Phe Gly Ile Leu Thr Arg Asp Phe Ser  
85 90 95  
Ser Thr Gly Trp Asn Ser Thr Thr Glu Asp Thr Ile Val Pro His Leu  
100 105 110  
Gly Lys Tyr Tyr Phe Asn Leu Met Gln Tyr Leu Glu Ser Lys Glu Tyr  
115 120 125  
Asp Arg Cys Ala Trp Thr Val Val Gln Val Gln Ile Leu Thr Asn Val  
130 135 140  
Ser Phe Leu Met Arg Leu Thr Gly Tyr Val Arg Asp  
145 150 155

<210> 21

&lt;211&gt; 166

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 21

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln  
 1 5 10 15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu  
 20 25 30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln  
 35 40 45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln  
 50 55 60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn  
 65 70 75 80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn  
 85 90 95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr  
 100 105 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg  
 115 120 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr  
 130 135 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu  
 145 150 155 160

Thr Gly Tyr Leu Gly Asn  
 165